UNIVERSITY OF EDINBURGH

A THESIS submitted

by

JOHN MUNRO, B.Sc.

a candidate to qualify

for the degree

of

DOCTOR OF PHILOSOPHY

May 1936

Title.

Investigations on Substitution in the Sugar Group.

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C O N T E N T S.

Investigations on Substitution in the Sugar Group.

In the early days of organic chemistry the carbohydrates offered great difficulties to investigators, and little progress was made in their study until Fischer discovered the crystalline phenylhydrazones and phenylosazones of the sugars. In an incredibly short time he carried to a successful conclusion a vast research which settled many problems in the field of the monosaccharides. But, in the period following this, interest in sugar chemistry suffered what was, perhaps, a natural decline, by comparison with the brilliant Fischer era, and it was not until a new method of approach had been devised that carbohydrate chemistry came to the fore again. Purdie and his collaborators (1) were the pioneers, and their new instrument of attack was the process of methylation. They showed that sugars and glycosides could be alkylated by treatment with an alkyl iodide in contact with dry silver oxide. This method, however, is applicable only to carbohydrates for which a suitable solvent can be found; and, as the silver oxide has an oxidising action on sugars containing a free reducing group, it is usually necessary to prepare the methyl-glycoside

and utilise it for methylation, instead of the free sugar. The process is, moreover, slow and expensive. Haworth (2) later demonstrated how methylation could be more readily accomplished by using methyl sulphate and aqueous sodium hydroxide, and this method enabled rapid progress to be made in the elucidation of the ring structures of the monosaccharides, and in the assignment of constitutions to the more complex carbohydrates.

By the above methods, methylated glycosides are produced, hydrolysis of which, with dilute mineral acids, leads to the elimination of the glycosidic methyl residue with the formation of the methylated free sugars. The remaining methyl groups are relatively stable and can withstand the action of dilute acids and alkalis, a fact which makes possible the identification of the hydrolytic break-down products of the methylated polysaccharides. During methylation, so far as is at present known, stereochemical changes, such as racemisation, Walden inversion or change in ring structure do not take place, and it is on this account that methylation plays such an important role in the field of carbohydrate chemistry. Acyl groups, however, sometimes have a tendency to wander during the reaction, and this possibility has always to be con-

sidered.

In recent years remarkable progress has been made, chiefly by Haworth, Hirst, and their co-workers, in the elucidation of the structures of the mono-, di- and polysaccharides. Di- and polysaccharides, on hydrolysis, split up into monoses which can be readily identified. Methylated di- and polysaccharides also split up, on hydrolysis, giving methylated monoses, which need to be identified before the final structure of the original compound can be determined. It is, therefore, essential that the list of methylated reference compounds at the disposal of the investigator should be as complete and reliable as possible.

Degradation products of methylated di- and polysaccharides are usually tri- and tetramethyl monoses, and most of these are now well known. On the other hand, very few mono- and dimethyl derivatives have been isolated and definitely identified, and the constitutions ascribed to some of these are still open to criticism. These partly methylated sugars and their derivatives, usually crystalline phenylhydrazones or phenylosazones, are required as reference compounds for the assignment of structure to the isopropylidene, and the alkali addition compounds of the carbohydrates.

Whereas well characterised derivatives of 2-, 3-

and 6-methyl glucose were already available, there was dubiety about the existence of such reference compounds for 4- and 5-methyl glucose, and this dubiety extended to the reference compounds of 4-methyl galactose and 4-methyl mannose, since the methods of preparation and identification of these compounds appeared to be inter-dependent. It was, therefore, considered desirable to remove all doubt on these points.

P A R T I.

The Constitution of the Supposed 4-Methyl Glucose.

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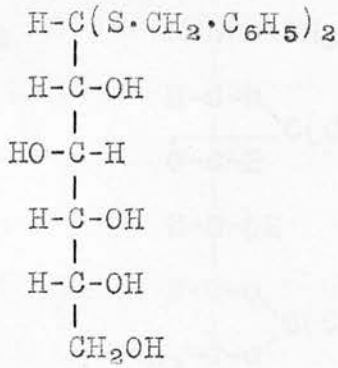
The isopropylidene compounds of the monosaccharides may be used for synthesising mono- and tri-substituted sugars by introducing various acyl or alkyl residues into the free hydroxyl groups, and removing the acetone residues by hydrolysis with dilute acid. These acetone sugars, however, always lead to derivatives substituted in the same positions, and to obtain new isomers, the acetone residues in the molecule would have to be situated in positions different from those occupied in the ordinary isopropylidene derivatives of the sugars. Such compounds would be available if it were possible to condense with acetone true straight chain derivatives of monosaccharides, containing no oxide ring.

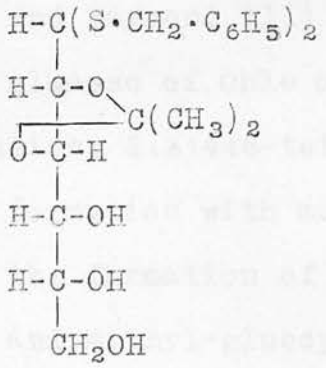
In 1924, a Hungarian chemist, Pacsu (3) employed such a compound for the preparation of new methylated glucose derivatives. As his starting material he used glucose dibenzyl mercaptal (I), prepared by condensing glucose with benzyl mercaptan in presence of concentrated hydrochloric acid.

(I)

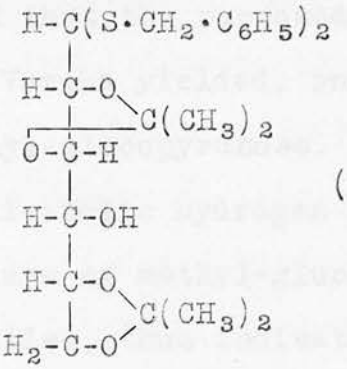


This derivative, on condensation with acetone in the presence of concentrated sulphuric acid, yielded a mixture of mono- and diacetone compounds, which, on methylation with sodium and methyl iodide and subsequent removal of acetone, gave two crystalline methylated mercaptal compounds, one a monomethyl and the other presumed to be a trimethyl glucose dibenzyl mercaptal. Hydrolysis of the mercaptan residues (4) yielded the corresponding free sugars. Having prepared an osazone from each of these methyl glucoses, Pacsu concluded that position 2 was not methylated, and that an acetone group must have been attached to the 2:3- position (II). Since the monomethyl derivative differed from the known 2- and 3-methyl glucoses and also from Helferich's 6-methyl glucose (5), the second acetone residue could have been attached only at positions 5 and 6 (III). From these suppositions Pacsu came to the conclusion that the monomethyl derivative was 4-methyl glucose and the trimethyl derivative, 4:5:6-trimethyl glucose.

(II)



(III)



This work was, however, challenged by Schinle (6) who revealed that the supposed 4-methyl glucosazone was, in reality, impure glucosazone, an observation which led to the conclusion that the parent monomethyl sugar was 2-methyl glucose, identical with that previously described by Hickinbottom (7) and by Brigl and Schinle (8). Schinle followed up these observations (9) by a study of the 4:5:6-trimethyl glucose reported by Pacsu, and found it to be in fact a monomethyl glucose, yielding a monomethyl glucose phenylosazone, m.p. 160° (Cf. Pacsu (4), m.p. 157°). The melting point and specific rotation of the osazone, and the specific rotation of the original sugar, differentiated the latter from the known 2-, 3- and 6-methyl glucoses, as well as from the supposed 5-methyl glucose of Ohle and v. Vargha (10). Schinle accordingly designated this sugar 4-methyl glucose, but, unfortunately, he carried out no conclusive experiments to confirm this characterisation.

The position became indefinite once more when

Levene and Raymond (11) showed that the supposed 5-methyl glucose of Ohle and v. Vargha yielded, on methylation, 2:3:4:6-tetramethyl glucopyranose. Glycoside formation with methyl-alcoholic hydrogen chloride led to the formation of a mixture of methyl-glucofuranosides and methyl-glucopyranosides, thus indicating that positions 4 and 5 were free, and Levene and Raymond regarded the supposed 5-methyl glucose as identical with 6-methyl glucose. This view was supported when Helferich (12) corrected some of the constants he had previously quoted for 6-methyl glucose. Obviously, when these new facts came to light, Schinle's method of assignment of position 4 to the methyl sugar under review did not exclude a selection of position 5. As was to be expected, Levene and Raymond (13) re-examined the question and adduced indirect evidence supporting the original conclusion of Schinle (9), but a survey of this evidence reveals the fact that it, too, is not entirely conclusive.

Instead of a critical examination of the "4:5:6-trimethyl glucose" of Pacsu (4), Levene and Raymond (13) synthesised a crystalline compound described as 2:3:6-triacetyl 4-methyl β-methylglucoside, and compared it with the corresponding derivative prepared directly, through the acetobromo-compound from the sugar in

question. The stages of their synthesis were : β-methylglucoside (I) ⟶ 4:6-benzylidene β-methylglucoside (II) ⟶ 2:3-dibenzoyl 4:6-benzylidene β-methylglucoside (III) ⟶ 2:3-dibenzoyl β-methylglucoside (IV) ⟶ 2:3:6-tribenzoyl β-methylglucoside (V) ⟶ 2:3:6-tribenzoyl 4-methyl β-methylglucoside (VI) ⟶ 4-methyl β-methylglucoside (VII) ⟶ 2:3:6-triacetyl 4-methyl β-methylglucoside (VIII). Levene and Raymond accept the view of Ohle and Spencker (14) that (II) has a pyranose form and argue, therefore, that positions 2 and 3 are available for benzoylation. They consider that, since (VIII) is originally derived from a normal glucoside, it cannot be substituted in position 5. But it is clear that no direct evidence is presented, at any rate after stage (II), that glucopyranosides are concerned. In addition, the possibility of the wandering of acyl groups during the methylation with methyl iodide and silver oxide was not considered, and although Helferich and Günther (12) record that 2:3:4-tribenzoyl β-methylglucoside passes on methylation into the corresponding 6-methyl derivative, it is well known that acetyl groups migrate during such treatment (15). Although the interpretation of Levene and Raymond was by no means improbable, it was considered that the question at issue was not decisively proved and it was determined to put the matter to a

critical test, from which the fact emerges that the 4-methyl glucose of Schinle has indeed that structure.

By a modified method, the syrupy methylated glucose of Schinle (9), i.e. the "4:5:6-trimethyl" glucose of Pacsu (4) was isolated, but all attempts at crystallisation failed. Complete methylation, followed by hydrolysis, yielded 2:3:4:6-tetramethyl glucopyranose in good yield, which thus excluded the possibility of the presence of 5-methyl glucose. Oxidation of the monomethyl glucose with bromine water yielded a monomethyl gluconolactone which, on account of its rapid hydrolysis in aqueous solution, was shown to be a δ-lactone. The inference is, therefore, that the possibility of the formation of the more stable γ-lactone was ruled out by the presence of a methyl group in the 4-position. Furthermore, complete methylation of the monomethyl δ-gluconolactone yielded 2:3:4:6-tetramethyl δ-gluconolactone, identified as the crystalline phenylhydrazide, thus supporting the view that the original oxidation product was indeed 4-methyl δ-gluconolactone.

In addition, Levene and Raymond's 2:3:6- triacetyl 4-methyl β-methylglucoside (13) was prepared from the monomethyl glucose by acetylation and glucoside formation through the acetobromo-compound, and it was found to have the properties ascribed to it by the American authors. Simultaneous deacetylation and

methylation, followed by hydrolysis, again yielded crystalline 2:3:4:6-tetramethyl glucopyranose.

From these results there appears no room for doubt that the monomethyl glucose, formerly supposed to be 4:5:6-trimethyl glucose, is in reality 4-methyl glucose.

EXPERIMENTAL.

Preparation of Glucose Dibenzyl Mercaptal.

The method of preparation described by Pacsu (3) was followed. To a solution of anhydrous zinc chloride (25 g.) in concentrated hydrochloric acid (50c.c.), anhydrous glucose (50 g.) was added, and the mixture was shaken until a homogeneous solution was obtained. Benzyl mercaptan (50 g.) was then introduced and the mixture shaken for 45 minutes, when it set to a greyish-white solid mass. This was broken up with a little water and filtered. Excess of benzyl mercaptan was removed by kneading well with benzene (300 c.c.), filtering and washing with more benzene (200 c.c.). The white solid was recrystallised, first from 3 litres of water, and finally from alcohol. Yield, 70 g. or 60% of theory. Glucose dibenzyl mercaptal crystallised in long, white needles, m.p. 139°.

Preparation of 2-Methyl Glucose Dibenzyl Mercaptal and 4-Methyl Glucose Dibenzyl Mercaptal.

Again the methods of Pacsu (3) and Schinle (9) were followed except for modifications of detail.

Glucose dibenzyl mercaptal (30 g.) was dissolved in dry acetone (300 g.), containing concentrated sulphuric acid (6 g.), and kept for 24 hours at 15°.

The solution, which had turned light brown in colour, was neutralised with anhydrous sodium carbonate and the acetone was removed by evaporation under diminished pressure. The acetone compound was obtained as a brownish, viscid syrup (20 g.). This derivative was dissolved in anhydrous ether (130 c.c.) and treated with excess of sodium shavings for 24 hours. After filtration and removal of the ether, the resulting glass was methylated with methyl iodide (35 c.c.) at 40° for 24 hours. During the treatment with sodium and the methylation, access of moisture was prevented by attaching a calcium chloride tube to the open end of the reaction vessel. The solution, on extraction with ether, filtration and evaporation, yielded a syrup which was dissolved in ten times its weight of 90% alcohol and hydrolysed by boiling for 20 minutes with N-hydrochloric acid (6 c.c.). From this solution 2-methyl glucose dibenzyl mercaptal, m.p. 191°, (i.e. the "4"-methyl glucose dibenzyl mercaptal of Pacsu) crystallised as needles on standing overnight at 0°. Yield, 3·5 g. or 20% of theory.

Analysis.

Found: OMe, 6·7. Calc. for $C_{21}H_{28}O_5S_2$, OMe, 7·3%.

All methoxyl analyses in this work were done by a method

based on the micro-Zeisel method of Pregl, suitably modified so that about 10 mg. of substance was used for a determination with compounds of methoxyl content 7-16%. For the analysis of compounds containing sulphur, the aqueous red phosphorus trap was replaced by a 5% cadmium sulphate suspension of red phosphorus, which converted hydrogen sulphide to insoluble cadmium sulphide.

The 4-methyl glucose dibenzyl mercaptal, i.e. the "trimethyl" glucose dibenzyl mercaptal of Pacsu, was obtained by the addition of water to the filtrate from the 2-methyl glucose dibenzyl mercaptal until a turbid solution was produced, from which on standing at 0°, a crystalline precipitate was deposited. This was dissolved in alcohol, treated with silver carbonate to remove hydrochloric acid, and decolorised with animal charcoal. Concentration yielded plates of 4-methyl glucose dibenzyl mercaptal, m.p. 73° on recrystallisation from ether (7 g. or 40% of theory).

Isolation of 4-Methyl Glucose.

For the removal of the mercaptan residue, 4-methyl glucose dibenzyl mercaptal (8 g.) was dissolved in 80%

aqueous acetone (100 c.c.), and a concentrated acetone solution of mercuric chloride (13 g.) was added. After boiling under reflux for an hour, the insoluble addition compound, $C_6H_5 \cdot CH_2 \cdot S \cdot HgCl$, was filtered off, and the acetone was removed by evaporation at 50°/20 mm. The syrup was dissolved in water, and the solution filtered from a further crop of the insoluble mercury compound. Excess of mercuric chloride was then removed by treatment with hydrogen sulphide, and the hydrochloric acid formed during the reaction was neutralised with silver carbonate. The solution, after filtration, was concentrated to a syrup (2·5 g.), but all attempts at crystallisation failed.

$[\alpha]_D^{20}$ + 53° (equil. value) in water (c, 2·1).

Analysis.

Found: OMe, 14·8. Calc. for $C_7H_{14}O_6$, OMe, 16·0%.

Preparation of 4-Methyl Glucose Phenylosazone.

The osazone was prepared in the usual way by dissolving 4-methyl glucose (0·1 g.) in water (0·5 c.c.), and heating for half an hour at 100° with phenylhydrazine (0·5 c.c.) and glacial acetic acid (0·5 c.c.). The

osazone crystallised on cooling, and recrystallisation from aqueous alcohol gave a compound, m.p. 158° (Cf. Pacsu (4); Schinle (9).).

Preparation and Identification of the Fully Methylated Glucose from 4-Methyl Glucose.

Tetra-acetyl 4-Methyl Glucose.

4-methyl glucose (1 g.) was dissolved in pyridine (4·5 c.c.) and acetic anhydride (4·5 c.c.) was slowly added. The solution was then heated to 50°, allowed to stand at room temperature for 36 hours and poured into ice-water (50 c.c.). The oil was extracted with ether, and ethereal solution was washed with dilute sulphuric acid, sodium bicarbonate solution, and finally with water. After drying over calcium chloride and removal of solvent, a yellow syrup was obtained. (1·5 g.)

Triacetyl 4-Methyl Glucosidyl Bromide.

To the above acetyl compound (1·5 g.), dissolved in glacial acetic acid (2 c.c.), glacial acetic acid

saturated with hydrogen bromide at 0° (3 c.c.) was added. After 2 hours, cold chloroform (15 c.c.) was added, and the mixture poured into ice-water (40 c.c.). The chloroform solution was washed with sodium bicarbonate solution and water, and dried, and the solvent removed at 45° (diminished pressure) to yield a pale yellow syrup (1·15 g.).

2:3:6-Triacetyl 4-Methyl β-Methylglucoside.

The acetobromo-compound (1·15 g.) was dissolved in dry methyl alcohol (20 c.c.), and shaken for 12 hours with dry silver carbonate (3 g.), until no bromide ions could be detected in the solution. This was filtered and evaporated to a thin syrup which crystallised on standing, and the long, colourless needles (0·7 g.) were washed free from syrup with alcohol. They showed m.p. 106°,

$[\alpha]_D^{20}$ - 34·0° in chloroform (c, 1·2).

Analysis.

Found: OMe, 16·9. Calc. for $C_{14}H_{22}O_9$,
OMe, 18·5%.

(Cf. Levene and Raymond (13).)

Methylation of 2:3:6-Triacetyl 4-Methyl β-Methylglucoside.

The triacetyl 4-methyl β-methylglucoside (0·5 g.),

dissolved in acetone (10 c.c.), was methylated in the usual way (2) with methyl sulphate (15 c.c.) and sodium hydroxide solution (40 c.c. of 30%), added in equal proportions during 1 hour, the temperature being maintained at 56-60°. After the final addition of reagents the temperature was raised to 75° for half an hour. The syrup obtained, after extracting with chloroform, washing with water and drying over calcium chloride, and removal of solvent, was methylated during 6 hours at 40° in contact with methyl iodide (10 c.c.) and dry silver oxide (4 g.). After extraction with ether and removal of solvent, the syrup yielded on distillation at 0·03 mm. tetramethyl methylglucopyranoside (0·3 g.) at 100° (bath temp.); $n_D^{15°}$ 1·4450.

2:3:4:6-Tetramethyl Glucopyranose.

The tetramethyl methylglucoside (0·3 g.) was hydrolysed with 5% hydrochloric acid (2 c.c.) for 8 hours at 80°. This was followed by neutralisation with barium carbonate and the solution was evaporated. After the addition of alcohol to precipitate most of the barium chloride, and filtration, the solid was extracted three times with boiling ether. The ethereal solution was evaporated, and the syrup extracted with boiling light petroleum-ether (60/80°). From this solution the characteristic crystals of 2:3:4:6-tetramethyl

glucose were deposited, m.p. 81-82° alone or in admixture with an authentic specimen. It was thus established that Levene and Raymond's 2:3:6-triacetyl 4-methyl β-methylglucoside yielded 2:3:4:6-tetramethyl β-methylglucoside on methylation.

Direct Methylation of 4-Methyl Glucose.

4-methyl glucose (0·35 g.), dissolved in acetone (10 c.c.), was methylated twice as before with methyl sulphate (10 c.c.) and sodium hydroxide solution (20 c.c. of 30%). During the first three additions the temperature was maintained at 30° in order to facilitate the initial formation of the glucoside. The product was extracted with chloroform and the chloroform removed by evaporation. The second methylation was followed by two treatments with methyl iodide (10 c.c.) and silver oxide (5 g.). The resulting syrup (0·15 g.) distilled at 100-110° (bath temp.)/0·03 mm. to yield a mobile, colourless liquid (0·07 g.), $n_D^{15°}$ 1·4445.

The glucoside was hydrolysed as before to yield 2:3:4:6-tetramethyl glucose, and this was recrystallised twice from light petroleum (60/80°); yield 0·04 g., m.p. 82-83° alone or in admixture with an authentic specimen of 2:3:4:6-tetramethyl glucose.

Analysis.

Found: OMe, 51·2. Calc. for $C_{10}H_{20}O_6$,
OMe, 52·5%.

Oxidation of 4-Methyl Glucose to 4-Methyl δ-Glucono-lactone.

4-Methyl glucose (0·8 g.) was oxidised in water (7 c.c.) with bromine (1·5 c.c.) at 35° for 3 days until all reducing action had ceased. The excess of bromine was then removed by aëration, and the solution neutralised with silver carbonate. To obtain the lactone, the silver was precipitated with hydrogen sulphide, and the solution, after filtration, was evaporated to dryness at about 80° under diminished pressure.

$[\alpha]_D^{20}$ + 54·6° (3 mins.); 47·0° (4 mins.); 39·5° (6 mins.);
+ 37·6°(15 mins.); 35·7° (50 mins.);
+ 33·9° (280 mins., constant value), in water (c, 0·5).

Analysis.

Found: OMe, 14·0. Calc. for $C_7H_{12}O_6$,
OMe, 16·1%.

Titration of the equilibrium solution with sodium hydroxide solution showed that the lactone had been hydrolysed to the extent of about 80%, 20·4 c.c. of 0·0088 N-

sodium hydroxide being required to neutralise the hexonic acid present at equilibrium, and 25·2 c.c. of the alkali being necessary for the complete neutralisation of the total acid.

From the hydrolysis curve and the titration figures it is evident that the lactone belongs to the δ-series (Cf. Haworth (16)), and that the methyl group is in the 4-position.

Methylation of 4-Methyl δ-Gluconolactone.

The lactone (0·4 g.) was dissolved in the minimum quantity of methyl alcohol and treated with methyl iodide (10 c.c.) and silver oxide (5 g.) during 20 hours at 40°. The lactone was extracted with methyl alcohol, evaporated to dryness, and the methylation repeated four times for shorter periods of 5 hours, when the product became completely soluble in methyl iodide; a final methylation in absence of methyl alcohol was then carried out. The syrup was distilled at 0·03 mm., the fraction distilling at 110-115° (bath temp.) being collected (0·15 g.).

This methylated lactone was digested with phenylhydrazine (0·5 g.) at 100° for 3 hours, and on extraction of the product with an ether-light petroleum mixture,

crystals of the phenylhydrazide of 2:3:4:6-tetramethyl gluconic acid were isolated, m.p. 114°. (Cf. (17))

Preparation of 2-Methyl Glucose from 2-Methyl Glucose Dibenzyl Mercaptal.

Pure 2-methyl glucose dibenzyl mercaptal (3 g.), m.p. 190-191°, was dissolved in 80% aqueous acetone (50 c.c.) and the mercaptal residue was removed, as described on page 14 for 4-methyl glucose dibenzyl mercaptal, by treating with mercuric chloride (6 g.), dissolved in acetone (10 c.c.). The syrupy 2-methyl glucose (1 g.) was obtained, and this, on standing, crystallised, m.p. 157°.

Preparation of 2-Methyl Glucose Phenylhydrazone.

The 2-methyl glucose (0·2 g.), dissolved in water (0·5 c.c.), was allowed to stand at room temperature for 12 hours with phenylhydrazine (0·5 g.) and glacial acetic acid (0·05 g.). The phenylhydrazone crystallised, and recrystallisation from water gave needles, m.p. 178°. (Cf. (7) and (8))

Preparation of Glucosazone from 2-Methyl Glucose.

When a mixture of 2-methyl glucose, water and phenylhydrazine, in the proportions given above for the phenylhydrazone preparation, was heated on a water-bath for 45 minutes with glacial acetic acid (0·5 g.), glucosazone was obtained, m.p. 200°.

Analysis. Found: OMe, Nil.

Summary.

1. The acetone compounds of glucose dibenzyl mercaptal, when methylated with sodium and methyl iodide, and hydrolysed to remove acetone, yield two crystalline monomethyl glucose dibenzyl mercaptals, (A) and (B).

2. On removal of the mercaptan residues, (A) gives a crystalline monomethyl glucose, m.p. 157°, from which a monomethyl glucose phenylhydrazone, m.p. 178°, and glucose phenylosazone can be prepared. The methyl group is situated, therefore, in position 2, in agreement with the results of Schinle (6).

3. A syrupy monomethyl glucose is obtained from (B), and this forms a monomethyl glucose phenylosazone, m.p. 158°, different from the known 3- and 6-methyl glucose phenylosazones. This sugar was identified as follows.

4. Methylation of this monomethyl glucose gives 2:3:4:6-tetramethyl glucopyranose, thus excluding substitution in position 5.

5. Oxidation with bromine water produces a monomethyl δ-gluconolactone, which, on methylation, yields 2:3:4:6-tetramethyl-δ-gluconolactone, character-

ised by its crystalline phenylhydrazide.

6. From the monomethyl glucose, 2:3:6-triacetyl 4-methyl β-methylglucoside, identical with that prepared by Levene and Raymond (13), is obtained.

From these facts, it is concluded that the monomethyl glucose, formed from (B), is 4-methyl glucose.

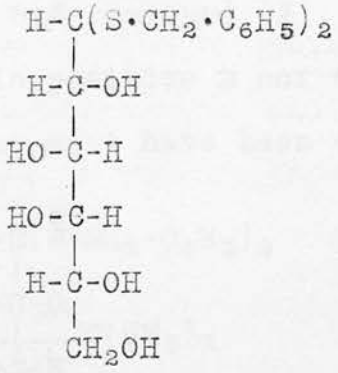
P A R T II.

A Revision of the Constitution of the Supposed 4-Methyl Galactose of Pacsu and Its Formulation as 6-Methyl Galactose.

A Revision of the Constitution of the Supposed 4-Methyl Galactose of Pacsu, and its Formulation as 6-Methyl Galactose.

In 1929 Pacsu and Löb (18) reported the isolation of a monomethyl galactose, obtained by a method similar to that employed for the preparation of the monomethyl glucoses, and they described it as 4-methyl galactose, apparently by analogy with the erroneously named glucose derivative (4), which was recognised eventually as 2-methyl glucose by Schinle (6).

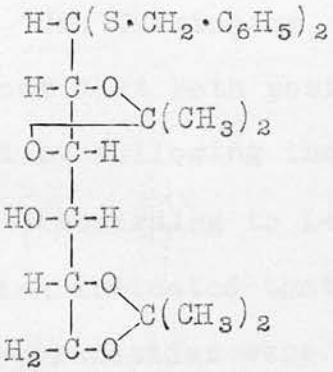
As their starting material Pacsu and Löb used galactose dibenzyl mercaptal, which has the formula,



and of which the acetone compound was made as before, and methylated with methyl sulphate and sodium hydroxide solution. Removal of acetone yielded a crystalline monomethyl galactose dibenzyl mercaptal, from which a

crystalline monomethyl galactose was isolated. The authors proceeded to make the osazone and found it to be a true monomethyl galactosazone, thus excluding substitution in position 2. Fairly close agreement was observed between the physical constants of the free sugar and its osazone, and those recorded by Freudenberg and Smeykal (19) for 6-methyl galactose and its osazone. Pacsu and Löb decided, however, that their methyl galactose was different, because on oxidation with silver oxide they were unable to obtain the silver salt of methoxy acetic acid ($CH_3O \cdot CH_2 \cdot COOAg$), which Freudenberg and Smeykal prepared from 6-methyl galactose by this method. Pacsu and Löb concluded, therefore, mainly by analogy with Pacsu's own previous experiments on glucose, that the acetone compound of galactose dibenzyl mercaptal, assumed to be a diacetone derivative, must have been the 2:3, 5:6-compound (I), since the methyl group was neither in position 2 nor 6, and that the monomethyl galactose must have been 4-methyl galactose.

(I)

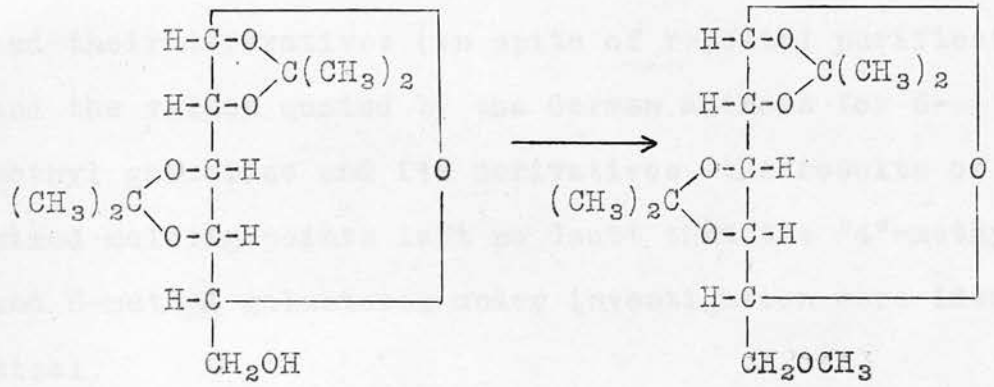


By methods essentially identical with those adopted by Pacsu and Löb, a quantity of the crystalline monomethyl galactose was prepared, and a preliminary examination of the phenylosazone indicated that it was a true monomethyl osazone, thus excluding substitution in position 2. 3-Methyl galactose was excluded by the melting point of its osazone (Robertson and Lamb (20)), so that only positions 4, 5 and 6 remained available for the assignment of the methoxyl residue. Complete methylation afforded 2:3:4:6-tetramethyl galactopyranose, isolated as the crystalline anilide. It is clear, therefore, that position 5 must be unsubstituted.

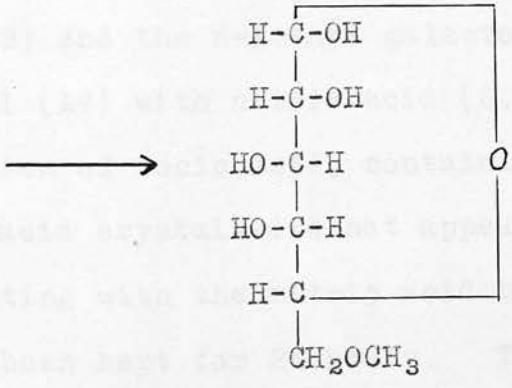
Confirmation that position 4 was also unmethylated was obtained by oxidation of the free sugar with bromine water, and the isolation of a monomethyl γ-galactonolactone, easily recognised by its negative specific rotation and slow rate of hydrolysis (16). In another experiment evidence was secured of the oxidation of the monomethyl galactose in the presence of sodium acetate to a δ-lactone. Cf. Carrington, Haworth and Hirst (21).

Further proof that both positions 4 and 5 were free was secured by following the progress of glycoside formation at 20°, according to Levene, Raymond and Dillon (22), which indicated that both galactofuranosides and galactopyranosides were formed. See Table II, page 50.

Throughout these investigations a marked similarity was observed between the physical constants of the free sugar and its derivatives, with those recorded by Freudenberg and Smeykal (19) for 6-methyl galactose, its phenylhydrazone and phenylosazone, of which the structures appear to be well established by the method of the isolation of 6-methyl galactose from diacetone galactopyranose.



(Diacetone Galactose) (6-Methyl Diacetone Galactose)



(6-Methyl Galactose)

Direct comparison of 6-methyl galactose and its derivatives, prepared as described by Freudenberg and Smeykal (19), with the corresponding compounds prepared

from the monomethyl galactose under investigation, led to the conclusion that the 4-methyl galactose of Pacsu was really 6-methyl galactose. See Table I, pages 32 and 33.

Although slight discrepancies existed between the melting points of "4"-methyl and 6-methyl galactose and their derivatives (in spite of repeated purifications), and the values quoted by the German authors for 6-methyl galactose and its derivatives, the results of mixed melting points left no doubt that the "4"-methyl and 6-methyl galactoses under investigation were identical.

Oxidation of the "4"-methyl galactose of Pacsu and Löb (18) and the 6-methyl galactose of Freudenberg and Smeykal (19) with nitric acid (S.G. 1·15) led to the formation of mucic acid, containing no methoxyl. The mucic acid crystals did not appear during the actual heating with the nitric acid but after the solution had been kept for 24 hours. This perhaps explains why Freudenberg and Smeykal failed to observe any mucic acid during their oxidation with nitric acid.

It is considered, therefore, that the "4"-methyl galactose of Pacsu and Löb must now be described as 6-methyl galactose.

Table I

		M.P.	Specific Rotation	Reference
1.	"4"-Methyl galactose	118°	$[\alpha]_D^{18}$ +117 ⟶ +68° (After 3 hrs. in water)	(a) Pacsu and Löb (18)
2.	"4"-methyl galactose	118-119°	$[\alpha]_D^{20}$ +120 ⟶ +70° (After 6 hrs. in water)	Prepared according to (a)
3.	6 -methyl galactose	122-123°	$[\alpha]_D^{20}$ +112 (4 mins.) ⟶ +66° (After 6 hrs. in water)	Prepared according to (b)
4.	6 -methyl galactose	128°	$[\alpha]_{578}^{20}$ + 114 (5 mins.) ⟶ +77° (After 3 hrs. in water)	(b) Freudenberg and Smeykal (19)
	Mixture of (2) & (3)	120°		
5.	"4"-methyl galactose phenylosazone	194-195°	$[\alpha]_D^{18}$ +131° (in pyridine)	(a) Pacsu and Löb (18)
6.	"4"-methyl galactose phenylosazone	200°	$[\alpha]_D^{18}$ +144° (in pyridine)	Prepared according to (a)
7.	6 -methyl galactose phenylosazone	200-201°	$[\alpha]_D^{20}$ +141° (in pyridine)	Prepared according to (b)
8.	6 -methyl galactose phenylosazone	204-205°	$[\alpha]_{578}^{20}$ +135° (in pyridine)	(b) Freudenberg and Smeykal (19)
9.	3 -methyl galactose phenylosazone.	176-179°		Robertson and Lamb (20)
	Mixture of (6) & (7)	200°		

Table I (contd.)

		M.P.	Specific Rotation	Reference
10.	"4"-methyl galactose phenylhydrazone	179°	$[\alpha]_D^{20}$ +24·4 ⟶ +14·1° (After 24 hrs. in pyridine)	Prepared as on page 39.
11.	6 -methyl galactose phenylhydrazone	179°	$[\alpha]_D^{20}$ +23·3 ⟶ +14·8° (After 24 hrs. in pyridine)	Prepared according to (b)
12.	6 -methyl galactose phenylhydrazone	182-183°	$[\alpha]_D^{20}$ +14·5° (in pyridine)	(b) Freudenberg and Smeykal (19)
	Mixture of (10) & (11)	179°		

EXPERIMENTAL.

Galactose Dibenzyl Mercaptal.

Galactose dibenzyl mercaptal was prepared as described by Pacsu and Tícharich (23). Galactose (50 g.) was dissolved in concentrated hydrochloric acid (50 c.c.) and shaken for 1-1½ hours with benzyl mercaptan (80 g.). The mixture solidified on standing overnight, and the solid was well kneaded with benzene to remove excess of benzyl mercaptan, filtered and washed with benzene, and dried on the filter. It was then dissolved in the minimum quantity of hot alcohol and allowed to crystallise. The crystals were washed with ether and recrystallised from alcohol. Yield, 45 g; m.p. 142-143°.

"4"-Methyl Galactose Dibenzyl Mercaptal.

The methods described by Pacsu and Löb (18), except for certain modifications of detail, were followed for the preparation. Galactose dibenzyl mercaptal (20 g.) was condensed with dry acetone (200 g.), containing concentrated sulphuric acid (6 c.c.), for 24

hours at room temperature. The acid was neutralised with anhydrous sodium carbonate, and the acetone compound (20 g.) was obtained as a yellow syrup after the removal of solvent at 50°/20 mm. This derivative was methylated in the usual way with methyl sulphate (20 c.c.) and sodium hydroxide solution (40 c.c. of 30%), added in equal proportions during 1 hour, acetone (30 c.c.) being used as a solvent. The temperature during the methylation was maintained at 56-60°, and after the final addition of reagents the temperature was raised to 75° for half an hour. The product, after pouring into cold water, was extracted with ether, and the ethereal solution washed with water and evaporated to a syrup. This was dissolved in ten times its weight of 80% alcohol, and hydrolysed by boiling for 15 minutes after the addition of concentrated hydrochloric acid (3 c.c.). The "4"-methyl galactose dibenzyl mercaptal was obtained by the addition of water until a turbid solution was produced, which on standing at 0°, gave place to a crystalline precipitate. The crystals (fine needles) were washed with a little cold alcohol and ether, and recrystallised from alcohol. Yield, 9g. or 53% of theory. They showed m.p. 130°, $[\alpha]_D^{20}$ -27° in pyridine (c, 3·3).

Analysis.

Found: C, 59·5 ; H, 6·7 ; OMe, 6·7.

$C_{21}H_{28}O_5S_2$ requires

C, 59·4 ; H, 6·6 ; OMe, 7·3%.

Carbon and hydrogen determinations were carried out using the Semi-Micro Combustion Apparatus described by Sucharda and Bobrański (24), and supplied by Greiner and Friedrichs.

Isolation of "4"-Methyl Galactose.

The "4"-methyl galactose dibenzyl mercaptal (10 g.) was dissolved in 80% aqueous acetone (200 c.c.), and the mercaptan residue was removed, precisely as described on page 14 for the isolation of 4-methyl glucose, by refluxing with a concentrated acetone solution of mercuric chloride (20 g.). The "4"-methyl galactose was obtained as a clear, viscous syrup (3·6 g.), which crystallised after several months. The crystals, after standing on porous tile in a desiccator, were washed free from syrup with an alcohol-ether mixture, and recrystallised from a little alcohol. They showed m.p. 118-119°, and $[\alpha]_D^{20}$ + 120 ⟶ + 70° (Constant value

after 6 hours) in water, (c, 1·4).

Analysis.

Found: C, 43·7 ; H, 7·4 ; OMe, 13·9.

$C_7H_{14}O_6$ requires

C, 43·3 ; H, 7·2 ; OMe, 16·0%.

"4"-Methyl Galactose Phenylosazone.

"4"-Methyl galactose (0·3 g.) was dissolved in water (1·5 c.c.), and heated on a water-bath with phenylhydrazine (1·5 g.) and glacial acetic acid (0·5 g.) for 1 hour. On cooling, the osazone crystallised, and recrystallisation from alcohol gave yellow needles, m.p. 200°, and $[\alpha]_D^{20}$ + 144° in pyridine (c, 0·4), the solution showing no mutarotation.

Analysis.

Found: OMe, 7·9 ; N, 15·1. Calc. for $C_{19}H_{24}O_4N_4$,

OMe, 8·3 ; N, 15·0%.

Nitrogen estimations were carried out with the Micro-Dumas apparatus described by Pregl, 5-8 mg. of substance being used for a determination with compounds

of Nitrogen content, 4-15%.

"4"-Methyl Galactose Phenylhydrazone.

As some difficulty was experienced in obtaining crystalline "4"-methyl galactose phenylhydrazone, several experiments were done with galactose to discover the best conditions for the preparation of galactose phenylhydrazone. The following mixtures were made and allowed to stand at room temperature for 12 hours, when it was found that the fifth mixture, containing galactose, water and phenylhydrazine, was the only one to give crystalline galactose phenylhydrazone.

(1) Galactose (0·3 g.), dissolved in water (1 c.c.); phenylhydrazine (0·6 g.); glacial acetic acid (0·1 c.c.)

(2) Galactose (0·3 g.), dissolved in water (1 c.c.); phenylhydrazine (0·6 g.); glacial acetic acid (0·1 c.c.); 2N-hydrochloric acid (0·05 c.c.).

(3) Galactose (0·3 g.), dissolved in water (1 c.c.); phenylhydrazine (0·6 g.); sodium acetate (0·5 g.); 2N-hydrochloric acid (0·1 c.c.).

(4) Galactose (0·3 g.), dissolved in water (1 c.c.); phenylhydrazine (0·6 g.); sodium acetate (0·5 g.); glacial acetic acid (0·1 c.c.).

(5) Galactose (0·3 g.), dissolved in water (1 c.c.); phenylhydrazine (0·6 g.).

"4"-Methyl galactose phenylhydrazone was accordingly prepared by dissolving "4"-methyl galactose (0·2 g.) in the minimum quantity of water and adding phenylhydrazine (0·5 g.). On mixing, a clear solution was formed, which began to crystallise after standing for a few hours at room temperature, crystallisation being complete in 2 days. The phenylhydrazone was recrystallised from methyl alcohol, and the crystals showed m.p. 179°, and $[\alpha]_D^{20}$ + 24·4° ⟶ 14·1° (24 hours, constant value) in pyridine (c, 0·9).

Analysis.

Found: OMe, 10·2 ; N, 9·8. Calc. for $C_{13}H_{20}O_5N_2$, OMe, 10·9 ; N, 9·85%.

Preparation and Identification of the Completely Methylated Galactose from "4"-Methyl Galactose.

In preparing the fully methylated galactose a series of reactions, similar to that previously used for the complete methylation of 4-methyl glucose (page 16), was employed, in the hope that some intermediate crystalline product would be obtained, but without success.

Tetra-acetyl "4"-Methyl Galactose.

"4"-Methyl galactose (1·6 g.) was dissolved in pyridine (8 c.c.) and acetic anhydride (8 c.c.) was slowly added. The solution was then heated to 50°, allowed to stand at room temperature for 24 hours and poured into ice-water (100 c.c.). The oil was extracted with ether, and the ethereal solution was washed with dilute sulphuric acid, sodium bicarbonate solution, and finally with water. After drying over sodium sulphate and removal of solvent, a yellow syrup was obtained (2 g.).

Triacetyl "4"-Methyl Galactosidyl Bromide.

To the above acetyl compound (2 g.), dissolved in glacial acetic acid (3 c.c.), glacial acetic acid saturated with hydrogen bromide at 0° (5 c.c.) was added. After

2 hours, cold chloroform (15 c.c.) was added, and the mixture poured into ice-water (50 c.c.). The chloroform solution was washed with sodium bicarbonate solution and water and dried over sodium sulphate, and the solvent removed at 45° (diminished pressure) to yield a yellow syrup (1·9 g.).

Triacetyl "4"-Methyl Methylgalactoside.

The acetobromo-compound (1·9 g.) was dissolved in dry methyl alcohol (25 c.c.), and shaken for 12 hours with dry silver carbonate (4-5 g.), until the solution gave no reaction for bromide ions with silver nitrate solution. The insoluble silver salts were filtered off, and the solution evaporated to a thin syrup (1·4 g.) which failed to crystallise.

Methylation of Triacetyl "4"-Methyl Methylgalactoside.

Triacetyl "4"-methyl methylgalactoside (1·4 g.), dissolved in acetone, was methylated in the usual way (2) with methyl sulphate (15 c.c.) and sodium hydroxide solution (40 c.c. of 30%) at 56-60°. The product was extracted with chloroform and the chloroform removed by evaporation. The syrup so obtained was remethylated twice with methyl iodide (10 c.c.) and dry silver oxide (2-3 g.) during 6 hours. After extraction with ether

and removal of the solvent, the syrup yielded on distillation tetramethyl methylgalactopyranoside (0·45 g.) at 115° (bath temp.)/0·045 mm., n_D^{15} 1·4500.

Hydrolysis to 2:3:4:6-Tetramethyl Galactopyranose.

The galactoside (0·44 g.) was heated on a water-bath at 80° with hydrochloric acid (4 c.c. of 8%) for 2 hours (25). The acid was neutralised with barium carbonate and, after filtration, the solution was evaporated to dryness under diminished pressure. Extraction of the solid with boiling ether yielded a clear syrup (0·4 g.), which failed to crystallise on standing for some weeks.

2:3:4:6-Tetramethyl Galactose Anilide.

The syrupy tetramethyl galactose (0·15 g.) was digested with aniline (0·4 g.) and alcohol (1 c.c.) at 100° for 3 hours. The anilide crystallised in long needles on cooling. Excess aniline was removed by distilling in a vacuum, and the residual solid (0·1 g.) was recrystallised from alcohol. It showed m.p. 192-193°, unchanged on admixture with an authentic specimen of 2:3:4:6-tetramethyl galactose anilide, prepared directly from galactose.

$[\alpha]_D^{20}$ - 71°, immediately on dissolution in acetone (c, 0·2).

Analysis.

Found: OMe, 40·8 ; N, 4·5. Calc. for $C_{16}H_{25}O_5N$, OMe, 39·9 ; N, 4·5%.

Oxidation of "4"-Methyl Galactose to "4"-Methyl γ-Galactonolactone.

"4"-Methyl galactose (1·5 g.), dissolved in water (10 c.c.), was oxidised with bromine (3 c.c.) at 35° until all reducing action had ceased (48 hours). The excess of bromime was removed by aëration, and the solution neutralised with silver carbonate. The lactone (1 g.) was obtained by precipitating the silver with hydrogen sulphide, and the solution, after filtration, was evaporated to dryness under diminished pressure, and heated at 100° for 2 hours in a vacuum.

$[\alpha]_D^{20}$ - 43° (5 mins.); - 42·3° (2 days); - 40·1° (6 days); - 39·5° (9 days, constant value) in water (c, 0·66).

Analysis.

Found: OMe, 14·4. Calc. for $C_7H_{12}O_6$, OMe, 16·1%.

From the hydrolysis curve it is evident that the lactone belongs to the γ-series and that the methyl group is not in the 4-position. The equilibrium value, - 39·5°, agrees fairly well with the equilibrium value, - 40·2°, reported by Freudenberg and Smeykal (19) for the specific rotation of 6-methyl galactonic acid in water at 18°.

Titration of a portion of the equilibrium solution (3 c.c. containing 0·0203 g. of the methyl galactonic acid) with 0·01 N-sodium hydroxide gave the following values.

(a) Volume of 0·01 N-sodium hydroxide necessary to neutralise the free methyl galactonic acid in the equilibrium solution = 1·45 c.c.

(b) Volume of 0·01 N-sodium hydroxide necessary to neutralise the total methyl galactonic acid in the solution = 9·82 c.c.

The percentage hydrolysis at equilibrium is, therefore, only 14·7%, indicating the presence of a γ-lactone.

Oxidation of "4"-Methyl Galactose, and the Production of a δ-Lactone.

At the first attempt to prepare "4"-methyl galactose, by the method described above (p. 36), a very low analytical value for methoxyl was obtained (OMe, 10% instead of 16%). The reason was ultimately traced to some sodium carbonate, present in the silver carbonate used for the final neutralisation of mineral acid, but at the time it was thought that the discrepancy was due to free galactose. It was decided to attempt a separation by acetylation and distillation in a high vacuum.

Acetylation. To a solution of "4"-methyl galactose (4 g.) in pyridine (20 c.c.), acetic anhydride (20 c.c.) was slowly added. It was at this stage that the sodium carbonate was discovered. Effervescence was noted and a crystalline precipitate, which proved to be sodium acetate, was deposited. The filtered solution was heated to 50° and then allowed to stand at room temperature for 24 hours. The mixture was poured into ice-water (100 c.c.), and the oil extracted with ether. The ethereal layer, after washing with dilute sulphuric acid, sodium bicarbonate solution and water, was dried over sodium sulphate and evaporated to a syrup.

Analysis.

Found: OMe, 7·8. Calc. for tetra-acetyl monomethyl galactopyranose, $C_{15}H_{22}O_{10}$, OMe, 8·5%.

The results of this acetylation indicated that the original assumption was incorrect, and that the low analytical value for the methyl galactose was due, not to free galactose, but to inorganic material.

Deacetylation. Methyl galactose tetraacetate (3 g.), dissolved in chloroform (10 c.c.), was deacetylated, according to the method of Zemplén (26), with a solution of sodium (0·1 g.) in cold, dry methyl alcohol (10 c.c.). After standing for 1 hour at about 0°, water (10 c.c.) and glacial acetic acid (0·4 c.c.) were added, and the chloroform layer separated. The aqueous layer was washed with chloroform, and the combined chloroform washings were washed with water. This water was added to the original aqueous extract, and the solution evaporated to a syrup (1·5 g.). The "4"-methyl galactose obtained in this way always contains sodium acetate.

Oxidation to the Lactone. The "4"-methyl galactose

(1·5 g.), dissolved in water (8 c.c.), was oxidised to the lactone as before with bromine (3 c.c.) at 35° until all reducing action had ceased (48 hours). The lactone was formed by heating for 1 hour at 100° in a vacuum. It was found possible to separate the lactone from inorganic material by extracting with dry methyl alcohol, evaporating to dryness and heating again for about half an hour.

$[\alpha]_D^{20}$ + 14·9° (2½ mins.); + 10·6° (15 mins.)
+ 8·5° (3 hours, constant value), in water (c, 0·5).

The rapid initial hydrolysis appears to indicate the presence of a δ-lactone. A possible explanation of this interesting result was found in the experiments of Carrington, Haworth and Hirst (21), where they show that sodium acetate tends to favour the production of 2:3:6-trimethyl δ-gluconolactone. A considerably higher rotation would have been expected, however, for pure monomethyl δ-galactonolactone, and it is thus highly probable that the γ-lactone, $[\alpha]_D^{20}$ - 40°, was also present in the mixture.

This result is a further indication that position 5 in the original methyl galactose is unsubstituted.

Glycoside Formation with 4-Methyl Galactose at 20°.

The method employed for examining the rate of glycoside formation at room temperature was essentially that described by Levene, Raymond and Dillon (22). A reducing sugar, when treated with methyl alcoholic hydrogen chloride, is converted into the glycoside. If positions 4 and 5 are unsubstituted, then the less stable furanoside structure is first formed, and this is slowly transformed to the more stable pyranoside structure. The furanosides can be hydrolysed to the reducing sugars by a dilute mineral acid, which does not affect the more stable pyranosides, and this gives a means of estimating approximately the amount of furanoside or pyranoside to which any reducing sugar gives rise on glycoside formation. The free reducing sugar is estimated either by the Hagedorn-Jensen or by a micro-modification of the Willstätter hypoiodite method. In the following experiments the hypoiodite method was employed.

From a 0·5% methyl alcoholic hydrogen chloride solution, containing approximately 6 mg. of "4"-methyl galactose per c.c., two samples of 0·5 c.c. were withdrawn at intervals.

One sample was treated with 0·4 N-sodium carbonate solution (0·5 c.c.), and water (3 c.c.) and allowed to

stand for 15 minutes with 0·3 N-sodium hydroxide (1 c.c.) and 0·03 N-iodine (5 c.c.). The excess iodine was then liberated with 5 N-sulphuric acid (0·2 c.c.) and titrated with 0·01 N-sodium thiosulphate.

The second sample was heated for 10 minutes at 100° with water (2 c.c.) and 0·26 N-hydrochloric acid (1 c.c.). After immediate cooling, the acid was neutralised with the calculated amount of 0·4 N-sodium carbonate, and the solution allowed to stand for 15 minutes with 0·3 N-sodium hydroxide (1 c.c.) and 0·03 N-iodine (5 c.c.). The excess of iodine was determined as before.

Blank titrations, in absence of sugar, were carried out under similar conditions, and the difference between these values and the actual experimental titration values gave the figures for the reducing content of the glycosidic samples. A correction of 21% had to be made on the reducing values obtained after hydrolysis of the "4"-methyl methylgalactopyranoside under these conditions, this being determined in a separate experiment.

Table II.

Table II.

Time	c.c. of 0·01 N-sodium thiosulphate		% Free Sugar			% Free Sugar	% Furan-oside	% Pyran-oside
	Before hydrolysis	After hydrolysis	Before hydrolysis	After hydrolysis	Corrected % after hyd.			
0	2·13	2·39	100	100	100	100	—	—
15 mins.	1·68	2·46	78·9	102·9	100	78·9	21·1	—
30 "	1·60	2·31	75·1	96·6	95·8	75·1	20·7	4·2
1 hr.	1·38	2·24	64·8	93·7	92·1	64·8	27·3	7·9
2 "	1·05	2·10	49·3	87·8	84·8	49·3	35·5	15·2
4 "	0·78	1·93	36·6	80·8	76·0	36·6	39·4	24·0
24 "	0·20	1·40	9·4	58·6	48·2	9·4	38·8	51·8
48 "	0·11	1·52	6·6	63·6	54·5	6·6	47·9	45·5

The table shows that, at the end of 24 and 48 hours, there is a mixture of furanoside and pyranoside, the furanoside having been formed first, and later transformed to the pyranoside. Although this method is not claimed to be strictly quantitative, the results show beyond doubt that the methyl group in the mono-methyl galactose cannot be attached to positions 4 or 5.

Oxidation of "4"-Methyl Galactose to Mucic Acid.

Several different samples of the "4"-methyl galactose, when heated for 3 hours at 100° with nitric acid (S.G. 1·15, 5 c.c.), were oxidised to mucic acid. During heating the solution turned dark brown in colour and was eventually evaporated to a semi-solid syrup. This was almost completely soluble in water, but when kept for 24 hours, a white, insoluble crystalline precipitate appeared. This was filtered off and shown to be mucic acid, m.p. 215°.

Analysis.

Found: OMe, Nil.

A second crop of the precipitate formed on

further standing.

Freudenberg and Smeykal (19) failed to observe the formation of mucic acid, but this is perhaps explained by the length of time necessary for its appearance.

Oxidation of "4"-Methyl Galactose with Mercuric Oxide.

Oxidation of "4"-methyl galactose with yellow mercuric oxide, in presence of calcium carbonate, after Freudenberg and Smeykal (19), failed to produce crystalline 6-methyl galactonic acid. A syrup was formed which on further oxidation with nitric acid (S.G. 1·15), as above, yielded mucic acid.

The Preparation of 6-Methyl Galactose by the Methods of Freudenberg and Smeykal (19).

Diacetone Galactose.

Except for slight modifications, the method of Freudenberg and Doser (27) was employed. Finely powdered galactose (40 g.) was shaken for 48 hours with dry acetone (1000 c.c.), containing concentrated sulphuric acid (20 g.). The yellowish solution was filtered from unchanged galactose and neutralised with anhydrous sodium carbonate. The acetone was removed and the syrup distilled at 130-140°/0·2 mm. The distillate (15 g.) was a clear, viscid syrup.

Diacetone Galactose 6-Methyl Ether.

Diacetone galactose (14 g.), dissolved in anhydrous ether (35 c.c.), was treated for 15 hours at 40-45° with excess of sodium shavings. Unchanged sodium was then removed, and the syrup, obtained on evaporation, allowed to stand with methyl iodide (20 g.) for 12 hours

at 35°. The compound was extracted with ether from sodium iodide, and the syrup, obtained after removal of ether, was distilled at 1 mm. As some unchanged diacetone galactose was still present, the distillate (5 parts) was treated for 12 hours at 35° with dry pyridine (5 parts) and p-toluene sulphonyl chloride (2 parts). The mixture was extracted with ether, and the ethereal solution washed with dilute hydrochloric acid, sodium bicarbonate solution and water, and dried over potassium carbonate. On concentration, however, some p-toluene sulphonyl chloride, which had been present in excess, was deposited, and the diacetone galactose 6-methyl ether was extracted from this with light petroleum (b.p. 60/80°). Removal of the solvent and distillation at 120° (bath temp.)/0·1 mm. yielded 8 g. of a clear syrup.

6-Methyl Galactose.

Diacetone galactose 6-methyl ether (8 g.) was hydrolysed by boiling under reflux for 1½ hours with 1% sulphuric acid (100 c.c.). The acid was neutralised with barium carbonate and the solution evaporated under diminished pressure to a syrup which crystallised (4 g.). The crystals, freed from syrup by washing with an alcohol-ether mixture, were recrystallised from

alcohol; m.p. 122-123°, and

$[\alpha]_D^{20}$ + 112° (4 mins.)⟶+ 66° (after 6 hours), in water (c, 1·8).

Admixture with "4"-methyl galactose (m.p. 118-119°) showed m.p. 120°.

From this 6-methyl galactose a phenylosazone and a phenylhydrazone were prepared by methods similar to those employed for the corresponding derivatives of "4"-methyl galactose (p.37 & 39). The 6-methyl galactose phenylosazone showed m.p. 200-201°, and

$[\alpha]_D^{20}$ + 141° in pyridine (c, 1·4), without mutarotation.

A mixed melting point with this compound and "4"-methyl galactose phenylosazone showed no depression.

The 6-methyl galactose phenylhydrazone had m.p. 179°, and

$[\alpha]_D^{20}$ + 23·5⟶14·8° (after 24 hours) in pyridine (c, 1·0).

and admixture with "4"-methyl galactose phenylhydrazone also showed no depression in melting point.

Oxidation of 6-methyl galactose with nitric acid (S.G. 1·15) under the same conditions as those used for "4"-methyl galactose (page 51) again yielded mucic acid.

Summary.

1. The acetone compound of galactose dibenzyl mercaptal, when methylated with methyl sulphate and sodium hydroxide solution, and hydrolysed to remove acetone, yields a crystalline monomethyl galactose dibenzyl mercaptal.

2. On removal of the mercaptan residue a monomethyl galactose, m.p. 118-119°, is obtained and this forms a monomethyl galactose phenylosazone, m.p. 200°, different from the known 3-methyl galactose phenylosazone. A monomethyl galactose phenylhydrazone, m.p. 179°, is also produced.

3. Complete methylation of the monomethyl galactose affords 2:3:4:6-tetramethyl galactopyranose, characterised by its crystalline anilide, thus excluding substitution in position 5.

4. Oxidation of the monomethyl galactose with bromine water produces a monomethyl γ-galactonolactone, indicating that position 4 is also unsubstituted.

5. Furthermore, oxidation of the monomethyl galactose with bromine water, in presence of sodium acetate, produces a monomethyl galactonolactone, which has

a positive rotation, and a high initial rate of hydrolysis, indicating the presence of a δ-galactono-lactone.

6. Glycoside formation at 20° shows that the monomethyl galactose is converted into a mixture of methyl-galactofuranosides and methyl-galactopyranosides, thus giving further evidence that both positions 4 and 5 are free.

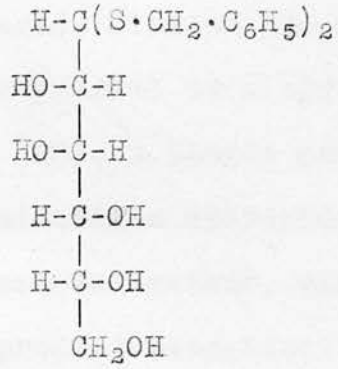
7. Direct comparison of the monomethyl galactose and its derivatives with 6-methyl galactose and its corresponding derivatives, prepared according to the method of Freudenberg and Smeykal (19), proves that the monomethyl galactose under investigation is indeed 6-methyl galactose.

P A R T III.

A Revision of the Constitution of the Supposed 4-Methyl Mannose of Pacsu and von Kary.

A Revision of the Constitution of the Supposed 4-Methyl Mannose of Pacsu and von Kary.

In a third series of experiments similar to those carried out with glucose and galactose, Pacsu and v. Kary (28) isolated a compound described as 4-methyl mannose from d-mannose dibenzyl mercaptal.



An examination of their paper reveals the fact that the osazone prepared from this 4-methyl mannose is identical with the osazone prepared from the supposed "4"-methyl glucose of Pacsu's first paper (4), and which was subsequently shown by Schinle (6) to be glucose phenylosazone itself. There is also no possibility that it is identical with true 4-methyl glucose phenylosazone, since this has widely different properties ((9), and page 15.).

If the osazone was really glucose phenylosazone it

is obvious, therefore, that the compound designated 4-methyl mannose, may be either mannose, 2-methyl mannose or a mixture of both. Owing to the fact that the original paper gave no analyses whatever for methoxyl, it was impossible to decide this point without repeating the work.

Mannose dibenzyl mercaptal was found to have the properties ascribed to it by the Hungarian authors. Condensation with acetone, in the presence of concentrated sulphuric acid, yielded the acetone compound of mannose dibenzyl mercaptal as a syrup. According to Pacsu and v. Kary (28), a single methylation with methyl sulphate and sodium hydroxide solution, followed by hydrolysis to remove acetone, caused the separation of a crystalline product described, on the basis of an analysis for sulphur, as 4-methyl mannose dibenzyl mercaptal, m.p. 188°, and $[\alpha]_D^{18}$ - 106·6° (in pyridine), together with a syrup.

Many attempts to reproduce this result failed. In every case an apparently homogeneous crystalline product, m.p. 118° and $[\alpha]_D^{20}$ approx. - 48° was obtained, but analysis invariably showed a methoxyl content of about one-third of the theoretical for a monomethyl mannose dibenzyl mercaptal. Furthermore, the melting point was not depressed in admixture with mannose

dibenzyl mercaptal itself (m.p. 126°), and it became evident that the compound was a mixture which could not be separated by repeated crystallisations, using various solvents. Attempts to separate the methylated and non-methylated portions by the removal of the mercaptan residue, glycoside formation, acetylation and distillation also failed.

By repeated acetone condensation, methylation and hydrolysis, it was found possible to increase the methoxyl content, and the specific rotation of the crystals, without, however, affecting the melting point, and in this way a crystalline product containing 80% of the theoretical amount of methoxyl for a monomethyl mannose dibenzyl mercaptal was obtained.

On the other hand, two consecutive methylations of the acetone compound with methyl sulphate and sodium hydroxide solution, without an intermediate hydrolysis to isolate the crystals of the methylated mannose dibenzyl mercaptal, failed to produce any increase in the methoxyl content.

Conversion of the methylated mannose dibenzyl mercaptal (methoxyl, 80% of theory) to the free sugar yielded a product which was still contaminated with free mannose, as was shown by the isolation of pure mannose phenylhydrazone in quantity corresponding with the amount of mannose present (20%), calculated from

the analytical results. No methylated phenylhydrazone could be isolated during many attempts, and, in the absence of any analysis for methoxyl in the original paper, the possibility cannot be excluded that the "4"-methyl mannose phenylhydrazone (m.p. 179°) of Pacsu and v. Kary (28) was mannose phenylhydrazone (m.p. 182-183°C).

After the removal of the mannose phenylhydrazone, heating produced three crops of an osazone of no methoxyl content, identical with glucose phenylosazone. The quantity of osazone was such that it must have been formed from that portion of the sugar containing methoxyl, and it can thus be stated with some certainty that the methoxyl group in the monomethyl mannose is located in position 2.

The results of Pacsu and v. Kary (28), however, do not depend on a study of the crystalline "4"-methyl mannose dibenzyl mercaptal, but are based on an examination of the sugar obtained on the simultaneous removal of the acetone and mercaptan residues from "2:3,5:6-diacetone 4-methyl mannose dibenzyl mercaptal". It was necessary, therefore, to examine the osazones and phenylhydrazones derived from the syrupy portion of the methylated mannose dibenzyl mercaptal in order to complete the evidence.

The acetone compound of mannose dibenzyl mercaptal

was methylated once as before. The syrup so obtained was hydrolysed with hydrochloric acid, and the crystalline, partly methylated mannose dibenzyl mercaptal (OMe, 2·8%) was separated as completely as possible, so that mannose phenylhydrazone from this source might be excluded. Removal of the mercaptan groups from the residual syrup yielded a free sugar, which had a slightly higher methoxyl content (OMe, 17·5%) than is required for monomethyl mannose (OMe, 16·0%). However, crystalline mannose phenylhydrazone was readily isolated in this case also, and an examination of the osazones produced on heating, after the removal of the mannose phenylhydrazone, revealed that they were specimens of glucose phenylosazone, contaminated with small amounts of methylated by-products.

It is considered, therefore, that the syrup investigated by Pacsu and v. Kary (28) was chiefly mannose and 2-methyl mannose together with some more highly methylated derivatives, and that the phenylosazone and phenylhydrazone, on which they based their conclusions, were glucose phenylosazone and mannose phenylhydrazone respectively.

EXPERIMENTAL.

Preparation of d-Mannose Dibenzyl Mercaptal.

Mannose (10 g.), dissolved in concentrated hydrochloric acid (10 g.), was shaken for 1 hour with benzyl mercaptan (14 g.). The mixture solidified after standing overnight, and the solid was kneaded well with benzene, filtered, washed with benzene, pressed on the filter and recrystallised from alcohol. The mannose dibenzyl mercaptal (12 g.) was shown to have the properties ascribed to it by Pacsu and v. Kary (28); m.p. 126°.

$[\alpha]_D^{20}$ - 32·6° in pyridine (c, 0·7).

Preparation of the Acetone Compound of Mannose Dibenzyl Mercaptal.

Mannose dibenzyl mercaptal (10 g.) was condensed with dry acetone (100 g.), in the presence of concentrated sulphuric acid (3 c.c.), the reaction being allowed to proceed for 24 hours at room temperature. After neutralisation with anhydrous sodium carbonate, the acetone solution was filtered and concentrated,

under diminished pressure, to a syrup, which was separated from unchanged original material by extraction with anhydrous ether. The acetone compound, after a preliminary heating for 2 hours at 100°/15 mm., was heated for 20 minutes at 110°/0·07 mm. The product showed

$[\alpha]_D^{20}$ + 79° in acetylene tetrachloride (c, 2·0).

Pacsu and v. Kary (28) quoted $[\alpha]_D^{20}$ + 66° in acetylene tetrachloride, but it was considered that their drying method (70° in a high vacuum) was inadequate, as certain acetone condensation products require a temperature higher than 70° for complete removal from a syrup.

Methylation of the Acetone Compound of Mannose Dibenzyl Mercaptal.

The acetone compound (20 g.) was heated to 60° with sodium hydroxide solution (8 c.c. of 30%). Methyl sulphate (20 c.c.) and sodium hydroxide solution (30 c.c. of 30%) were then added in equal proportions over an interval of 45 minutes, the temperature being maintained at 70-75°. After heating for a further 30 minutes at 75°, the mixture was poured into ice-water, and the oil extracted with ether. The ethereal layer was washed

with water, dilute ammonia solution, and again with water, and evaporated to a syrup. This syrup was dissolved in 80% aqueous alcohol (200 c.c.), and the acetone groups were removed by boiling for 10 minutes after the addition of concentrated hydrochloric acid (3 c.c.). Water was added and the turbid solution allowed to stand overnight at 0°. The white crystalline product (6·0 g.), which separated, was washed with water and recrystallised from alcohol.

The crystals, however, did not melt at 188°, as quoted by Pacsu and v. Kary (28), but at 118°, and several crops gave the same melting point. A mixed melting point with mannose dibenzyl mercaptal (m.p. 126°) showed a depression of 1°.

$[\alpha]_D^{20}$ – 48° in pyridine (c, 1·0).

Analysis.

Found: OMe, 2·4. Calc. for monomethyl mannose dibenzyl mercaptal, $C_{21}H_{28}O_5S_2$, OMe, 7·3%.

These results were twice confirmed by separate experiments, in which crystalline products were obtained, giving values for specific rotation and methoxyl analysis approximately similar to those quoted above.

Isolation of the Partly Methylated Mannose.

It was hoped that, by isolating the free sugar, it might be possible to separate the monomethyl mannose. Accordingly, the partly methylated mannose dibenzyl mercaptal (5 g; OMe, 2·4%), dissolved in 80% aqueous acetone (100 c.c.), was boiled under reflux with a concentrated acetone solution of mercuric chloride (10 g.), the free sugar being obtained as a syrup (2 g.), in exactly the same way as 4-methyl glucose was isolated from 4-methyl glucose dibenzyl mercaptal (p. 14).

Analysis.

Found: OMe, 5·2. Calc. for monomethyl mannose, $C_7H_{14}O_6$, OMe, 16·0%.

Attempted Separation of "4"-Methyl Mannose Derivatives.

Conversion to the Methylglycoside.

The partly methylated mannose mixture (OMe, 5·2%) was converted to the glycoside, according to the method of Bott, Haworth and Hirst (29). The reducing sugar

(1 g.) was boiled under reflux for 1 hour with 2% methyl alcoholic hydrogen chloride (10 c.c.) and allowed to stand at 0° for 2 days. As no crystalline α-methylmannopyranoside separated, the solution was neutralised with silver carbonate, filtered and evaporated to a syrup.(0·6 g.)

Acetylation of the Glycoside.

The glycosidic mixture (0·6 g.) was dissolved in pyridine (8 c.c.) and acetic anhydride (5 c.c.) was slowly added. The solution, after standing for 24 hours at room temperature, was poured into ice-water (100 c.c.), and the oil was extracted with chloroform. The chloroform layer was washed successively with dilute sulphuric acid, sodium bicarbonate solution and water, and dried over sodium sulphate. Evaporation yielded a syrup (0·75 g.) which distilled in one fraction at 180-190° (bath temp.)/0·07 mm. Analysis showed it to be a mixture of tetra-acetyl methylmannoside and monomethyl triacetyl methylmannoside. So that separation of the methyl mannose from free mannose by this method proved unsuccessful.

Analysis.

Found: OMe, 11·1. Calc. for tetra-acetyl methylmannoside, $C_{15}H_{22}O_{10}$, OMe, 8·5. and for, monomethyl triacetyl methylmannoside, $C_{14}H_{22}O_9$, OMe, 18·5%.

Efforts to Increase the Methoxyl Content of the Partly Methylated Mannose Dibenzyl Mercaptal.

Contrary to expectation, it was found that, if the acetone compound of mannose dibenzyl mercaptal was methylated as before and the syrupy methylated acetone compound, so obtained, immediately remethylated by the same method, the crystals produced on hydrolysis with dilute acid, always had approximately the same methoxyl content as the crystals isolated after a single methylation.

On the other hand, if the crystals and syrup, formed after the methylation and hydrolysis of the acetone compound of mannose dibenzyl mercaptal, were condensed again with acetone and concentrated sulphuric acid, and then remethylated and hydrolysed as before, the crystals from this second methylation were found

to have a higher methoxyl content and also a higher specific rotation.

By this method, starting with the acetone compound of mannose dibenzyl mercaptal (10 g.), methylating with methyl sulphate and sodium hydroxide solution at 70° and hydrolysing as before (p. 65), a sample of crystals separated after this first methylation showed, m.p. 118° and $[\alpha]_D^{20}$ − 41·7° in pyridine.

Analysis.

Found: OMe, 2·3. Calc. for $C_{21}H_{28}O_5S_2$, OMe, 7·3%.

The crystals and syrup were then thoroughly freed from solvent and condensed with ten times their weight of dry acetone, containing concentrated sulphuric acid (3 g. per 100 g. of acetone). The acetone compound, obtained after neutralisation of acid and removal of solvent, was again methylated and hydrolysed, and this time a sample of the crystals showed, m.p. 117° and $[\alpha]_D^{20}$ − 46° in pyridine. The process was repeated once again, when crystals (0·5 g.) were separated having m.p. 117°, and

$[\alpha]_D^{20}$ − 54° in pyridine (c, 0·5).

(5 c.c.), as before (p. 14). The sugar, obtained in the form of a reducing sugar, had

$[\alpha]_D^{20}$ + 4·3° in water (c, 1·7).

Analysis.

Found: OMe, 11·7. Calc. for $C_7H_{14}O_6$, OMe, 16·0%.

Phenylhydrazone Formation.

The syrupy methylated mannose (0·06 g.), dissolved in water (1 c.c.), was mixed with phenylhydrazine (0·5 g.) and glacial acetic acid (0·1 c.c.). In a few seconds a white crystalline precipitate appeared (this is characteristic of mannose phenylhydrazone), and it was filtered off. After half an hour a further crop was removed. The clear solution was then allowed to stand at room temperature for 2 days, but no more crystals formed.

The crystalline phenylhydrazone (0·02 g.) was washed with a little cold acetone and dried. It showed m.p. 182-183° alone, or in admixture with a specimen of

mannose phenylhydrazone prepared directly from mannose.

Analysis.

Found: OMe, Nil; N, 10·3. Calc. for $C_{12}H_{18}O_5N_2$, OMe, Nil; N, 10·4%.

If it be assumed that the phenylhydrazone formation from mannose is almost quantitative, then 0·02 g. of mannose phenylhydrazone would be produced from 0·013 g. of mannose. The monomethyl mannose is, therefore, present to the extent of 75-80%, which agrees with the amount calculated from previous analytical results, and it is in order to say that the phenylhydrazone came entirely from the mannose present in the mixture, and also that its separation removed practically the whole of the free mannose present.

Phenylosazone Formation.

The clear filtrate, after the separation of mannose phenylhydrazone, was heated for 1 hour at 100° with glacial acetic acid (0·1 g.) and a crystal of sodium bisulphite (to retard tar formation). This treatment produced an osazone (0·01 g.), which was filtered and washed with cold acetone. It showed m.p. 204°

alone, or in admixture with an authentic specimen of glucose phenylosazone.

Analysis. Found: OMe, Nil.

The osazone must, therefore, have been glucose phenylosazone.

The filtrate on further heating yielded a second and a third crop of osazone, neither of which contained methoxyl (Yield, Ca. 0·01 g.). Since mannose gives a 30% yield of osazone under these conditions, it is clear that the glucose phenylosazone, now isolated, must have been formed from that portion of the syrup containing methoxyl, thus indicating that the methyl group is located in position 2.

Examination of the Syrupy "2:3,5:6-Diacetone 4-Methyl Mannose Dibenzyl Mercaptal" of Pacsu and von Kary.

Pacsu and v. Kary (28), in the preparation of their "4"-methyl d-mannose, did not utilise the crystalline "4"-methyl mannose dibenzyl mercaptal (m.p. 188°), which they obtained, but took, instead, the impure

"2:3,5:6-diacetone 4-methyl mannose dibenzyl mercaptal" prepared by methylating the mannose dibenzyl mercaptal acetone compound with methyl sulphate and sodium hydroxide solution, and removed the acetone and mercaptan residues simultaneously. An investigation of this method was next carried out.

The acetone compound of mannose dibenzyl mercaptal (5 g.) was methylated once, as before (p. 65), with methyl sulphate (8 c.c.) and sodium hydroxide solution (12 c.c.) at 70-75°. The solution was poured into water and the oil extracted with ether. The syrup, obtained after removal of solvent, was dissolved in 90% alcohol (50 c.c.) and hydrolysed by boiling for 10 minutes with concentrated hydrochloric acid (2 c.c.). Addition of water caused the precipitation of a mixture of crystals and syrup, which was dissolved in absolute alcohol and allowed to crystallise. After the separation of the first crystalline deposit, the solution was concentrated to a syrup which partly crystallised. The crystalline material (total yield, 0·45 g.) was separated as completely as possible from the syrup (1·8 g.) with ether, and the crystals were shown to be similar to the mixtures of low methoxyl content previously described. They had m.p. 117°, and $[\alpha]_d^{20}$ − 48° in pyridine (c, 1·0).

Analysis.

Found: OMe, 2·8. Calc. for $C_{21}H_{28}O_5S_2$,
OMe, 7·3%.

Removal of the Mercaptan Residue from the Syrup.

The syrup (1·5 g.), when dissolved in aqueous acetone (100 c.c.) and treated as before (p. 14) with mercuric chloride (4 g.) in acetone (15 c.c.), yielded a reducing sugar (0·5 g.) having,

$[\alpha]_D^{20}$ + 9·6° in water (c, 3·0).

Analysis.

Found: OMe, 17·5. Calc. for $C_7H_{14}O_6$,
OMe, 16·0%.

Separation of Mannose Phenylhydrazone.

The reducing syrup (OMe, 17·5%) (0·4 g.), dissolved in water (1 c.c.), was treated with phenylhydrazine (1·5 g.) and glacial acetic acid (0·1 c.c.). Almost immediately a crystalline precipitate of mannose phenylhydrazone appeared, and after half an hour this was removed,

washed with cold acetone and dried. (Yield, 0·1 g.) It showed m.p. 183°.

Analysis.

Found: OMe, Nil; N, 10·6. Calc. for $C_{12}H_{18}O_5N_2$, OMe, Nil; N, 10·4%.

Phenylosazone Formation.

The clear filtrate from the mannose phenylhydrazone, after standing at room temperature for 12 hours, was heated for 1 hour at 100° with glacial acetic acid (0·4 g.) and a crystal of sodium bisulphite. This produced an osazone (0·05 g.), which had m.p. 180-185°, and OMe, ca. 2%. On recrystallisation from alcohol, it proved to be chiefly glucose phenylosazone contaminated with a small amount of methylated material. A second crop (0·02 g.; OMe, ca. 3%) was also isolated and shown to be similar to the first. Obviously, therefore, the syrup which Pacsu and v. Kary (28) had examined must have been composed of mannose, 2-methyl mannose and small amounts of polymethylated derivatives, and the osazone, which they isolated, was derived from either mannose or 2-methyl mannose.

Methylation of the Acetone Compound of Mannose Dibenzyl Mercaptal with Sodium and Methyl Iodide.

In a further attempt to prepare a crystalline monomethyl mannose dibenzyl mercaptal, the methylation method with sodium and methyl iodide, used in the glucose series (p. 13), was tried. The acetone compound (5 g.), dissolved in anhydrous ether (50 c.c.), was treated for 24 hours at room temperature with an excess of sodium shavings, in a flask with calcium chloride tube attached. A white precipitate of the sodium salt appeared, and the ether solution and the precipitate were separated from the excess of sodium by decantation and washing with ether. The precipitate was not present in sufficient quantity to be separated from syrup and methylated by itself, but it was observed that it dissolved up on concentration of the ether solution. So the solution was concentrated to a glass (4 g.), which was dissolved in methyl iodide (30 g.), and maintained at 40° for 24 hours. The compound was extracted from sodium iodide with ether, and the syrup, obtained after removal of the solvent, was dissolved in alcohol (50 c.c.) and heated for 10 minutes at 100° with 2N-hydrochloric acid (3 c.c.). Water was added and the turbid solution allowed to stand overnight at 0°, but only an oily syrup resulted, from which no crystalline compound could be isolated.

Summary.

1. The acetone compound of mannose dibenzyl mercaptal, when methylated with methyl sulphate and sodium hydroxide solution, and hydrolysed to remove acetone, yields a crystalline partly methylated mannose dibenzyl mercaptal.

2. On removal of the mercaptan residue a syrupy methylated mannose, containing about one-third of the theoretical amount of methoxyl for a monomethyl mannose, is obtained, and from this compound mannose phenylhydrazone and glucosazone are produced.

3. The methoxyl content of the partly methylated mannose dibenzyl mercaptal can be increased by repeated acetone condensation, methylation and hydrolysis, and by this means a syrupy methylated mannose, containing 80% of the theoretical amount of methoxyl for a monomethyl mannose, is secured.

4. This methylated mannose, on treatment with phenylhydrazine, produces mannose phenylhydrazone in quantity proportional to the amount of free mannose present. After the removal of the phenylhydrazone, heating yields glucosazone, the amount of which cannot be accounted for except on the assumption that it is

derived from the methylated mannose present, thus indicating that the methyl group must have been located at position 2.

5. A repetition of the experiments of Pacsu and v. Kary (28) shows that in all probability the compounds which they had examined were not "4"-methyl mannose dibenzyl mercaptal and "4"-methyl mannose, but 2-methyl mannose dibenzyl mercaptal and 2-methyl mannose contaminated with mannose dibenzyl mercaptal and free mannose respectively.

PART IV.

The Constitutions of the Acetone Compounds of Glucose, Galactose and Mannose Dibenzyl Mercaptals.

The Constitutions of the Acetone Compounds of Glucose, Galactose and Mannose Dibenzyl Mercaptals.

The final stage of this investigation was devoted to a study of the acetone compounds of glucose, galactose and mannose dibenzyl mercaptals. It was evident from the diverse nature of the monomethyl hexoses to which these compounds gave rise, that the latter could not be, as Pacsu had assumed in his three papers, mainly 2:3,5:6-diacetone derivatives. Owing to the number of acetone compounds theoretically possible and to the great difficulty in isolating these compounds in a state of purity, any hypotheses as to their constitutions must of necessity be speculative.

Accurate analysis of the acetone compounds was impossible because of the difficulty experienced in purifying the syrups and removing acetone condensation products. Carbon and hydrogen determinations were found to give inconsistent results, but acetone estimations, by a modification of Messinger's method (p. 94), were of more value. Analytical figures for the acetone content of the acetone compounds of glucose, galactose and mannose dibenzyl mercaptals, as well as for those of the acetone compounds of 2-methyl glucose, 4-methyl

glucose and 6-methyl galactose dibenzyl mercaptals, prepared by a similar method, are given in Table I (p. 83). It is significant that the monomethyl mercaptal derivatives appear to form only monoacetone compounds, but this does not necessarily imply that these derivatives were formed from monoacetone compounds in the first instance.

Table I.

Acetone Compounds of	% $CH_3CO \cdot CH_3$
Glucose dibenzyl mercaptal	13·9 - 14·3
Galactose dibenzyl mercaptal	16·8 - 17·4
Mannose dibenzyl mercaptal	18·5 - 19·1
Calc. for diacetone hexose dibenzyl mercaptal, $C_{26}H_{34}O_5S_2$	23·67
Calc. for monoacetone hexose dibenzyl mercaptal, $C_{23}H_{30}O_5S_2$	12·9
2-methyl glucose dibenzyl mercaptal	11·2
4-methyl glucose dibenzyl mercaptal	10·1 - 10·5
6-methyl galactose dibenzyl mercaptal	9·6 - 10·0
Calc. for monoacetone monomethyl hexose dibenzyl mercaptal, $C_{24}H_{32}O_5S_2$	12·5

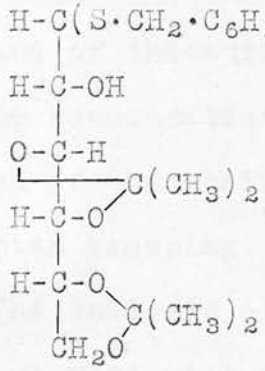
The Acetone Compounds of Glucose Dibenzyl Mercaptal.

The acetone compounds of glucose dibenzyl mercaptal, when methylated with sodium and methyl iodide and hydrolysed to remove acetone (p. 13), yielded 2-methyl glucose dibenzyl mercaptal (20%), 4-methyl glucose dibenzyl mercaptal (40%) and a syrup consisting of more highly methylated derivatives (p. 98).

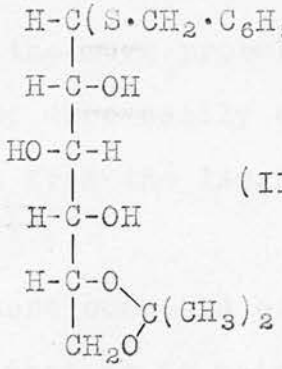
When glucose dibenzyl mercaptal was condensed with acetone, in the presence of anhydrous copper sulphate, it was shown that a crystalline monoacetone compound together with a syrup were produced (Pacsu (3); Schinle (9); and (p. 97).). Since Schinle reported that methylation of this crystalline monoacetone compound with sodium and methyl iodide yielded the 2-methyl glucose dibenzyl mercaptal, it might be supposed that the 20% yield of this compound arose from a monoacetone derivative in the mixture. Further evidence that position 2 in glucose dibenzyl mercaptal is very reactive is obtained from the work of Lieser and Leckzyck (30), who demonstrated that glucose dibenzyl mercaptal, dissolved in methyl alcohol, and methylated with silver oxide and methyl iodide, gave 2-methyl glucose dibenzyl mercaptal in 55% yield. It does not, however, follow that because position 2 in this compound is the most reactive to methylation, it will also be the most

reactive to acetone condensation. 2-methyl glucose dibenzyl mercaptal can theoretically be formed from only one diacetone compound (I), and also from any of the three monoacetone derivatives (II, III and IV).

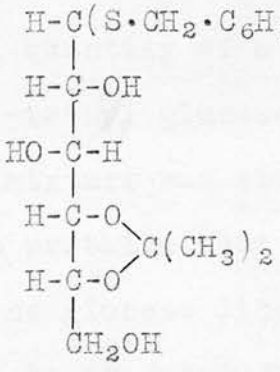
(I)



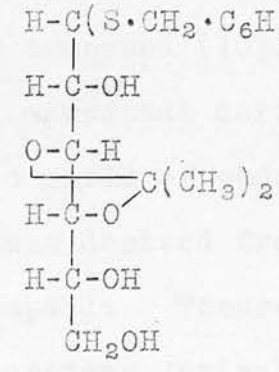
(II)



(III)



(IV)

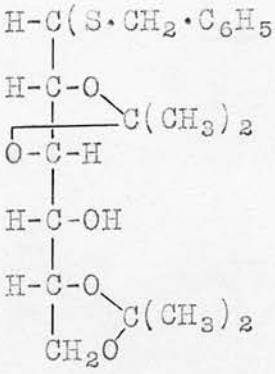


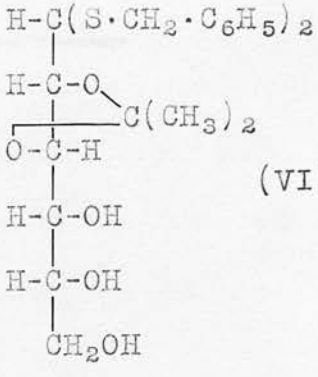
In considering the condensation of acetone with compounds containing a number of hydroxyl groups, it is usual to suppose that the acetone molecules will link up with two adjacent cis-hydroxyl groups. It is known, however, that linkage with trans-hydroxyl groups, in the case of acyclic derivatives, also occurs, but when a molecule contains adjacent cis-hydroxyls, condensation with

acetone would be expected to take place with these groups more readily than with hydroxyl groups in trans-positions. Structure (IV) above is therefore unlikely, and possibly also structure (I). A monoacetone compound will most probably have either structure (II) or (III), and of these (II) appears the more probable since acetone condensation should be more easily effected at the end of the chain farthest from the large benzyl mercaptan grouping.

The analysis of the acetone compound of glucose dibenzyl mercaptal indicates that it is mainly composed of a monoacetone derivative (90%), in admixture with a small quantity of a diacetone compound (10%). Since the 4-methyl glucose dibenzyl mercaptal derived from this mixture was obtained in a yield of about 40%, it seems probable that it, too, was derived from a monoacetone glucose dibenzyl mercaptal. Theoretically it might be produced from the diacetone derivative (V) or either of the two monoacetone derivatives (II) or (VI).

(V)





(VI)

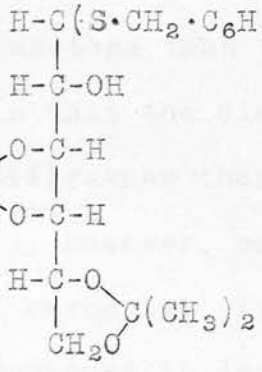
The possibility of the existence of the diacetone structure (V) cannot be ignored. If the monoacetone compound (II) were to form first, then it might reasonably be supposed that a second acetone molecule could link up with hydroxyls 2 and 3, as it has been shown that position 2 in the glucose dibenzyl mercaptal molecule is very reactive. The diacetone derivative (V) would give 4-methyl glucose dibenzyl mercaptal on methylation and hydrolysis, but the yield of the 4-methyl derivative appears to be too large to explain its formation from a diacetone compound in this way. On the other hand, as it has been assumed that 2-methyl glucose dibenzyl mercaptal was derived from a monoacetone derivative, it can be supposed that the 4-methyl compound was also derived from a similar source. Structures (II) and (VI) are possible for such a monoacetone compound, but again it appears more feasible that (II) should be formed in preference to (VI).

It is evident that, with the information at present available, finality cannot be reached on this point.

The Acetone Compounds of Galactose Dibenzyl Mercaptal.

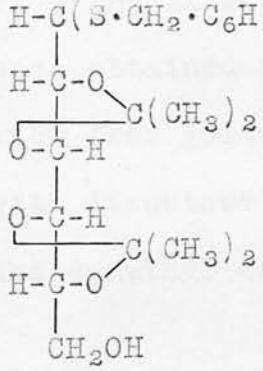
When the acetone compounds of galactose dibenzyl mercaptal were methylated with methyl sulphate and sodium hydroxide solution the 6-methyl derivative was produced in 50% yield (p. 35). This was contrary to expectation since it was considered that galactose dibenzyl mercaptal, on account of its configuration, would form the 3:4,5:6-diacetone compound (VII) and produce the 2-methyl derivative on methylation.

(VII)

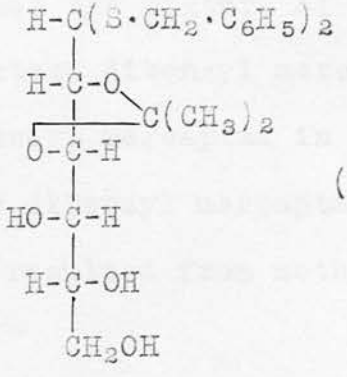


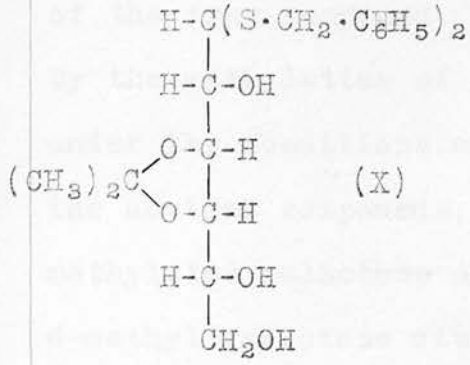
Theoretically the 6-methyl galactose dibenzyl mercaptal might have been formed from the diacetone derivative (VIII) or from any of the three monoacetone derivatives (IX, X, XI).

(VIII)

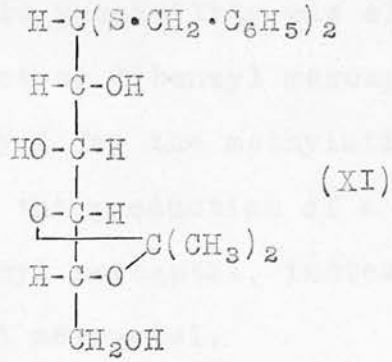


(IX)





(X)



(XI)

An examination of the analytical results (p. 83) shows that in all probability the syrup contained both mono- (60%) and diacetone (40%) derivatives. Assuming that cis-hydroxyl groups are more favourably placed for condensation with acetone than trans-hydroxyl groups, it is more probable that the diacetone compound in the mixture would be (VII) rather than (VIII). As the diacetone compound (VII), however, could not give 6-methyl galactose dibenzyl mercaptal, it is considered likely that this latter compound is derived from a monoacetone compound. Of the three possible isomers (IX, X and XI), (X) is to be favoured for the reasons expressed above, but a mixture of any of these compounds is possible.

It might be suggested that the mixture of acetone compounds obtained from galactose dibenzyl mercaptal contained free galactose dibenzyl mercaptal in admixture with diacetone galactose dibenzyl mercaptal, and that the 6-methyl derivative resulted from methylation

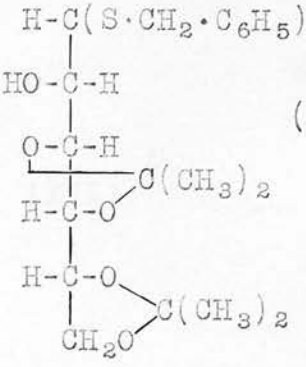
of the free compound. This possibility was eliminated by the methylation of galactose dibenzyl mercaptal, under the conditions employed for the methylation of the acetone compounds, and the production of a highly methylated galactose dibenzyl mercaptal, instead of 6-methyl galactose dibenzyl mercaptal.

The Acetone Compounds of Mannose Dibenzyl Mercaptal.

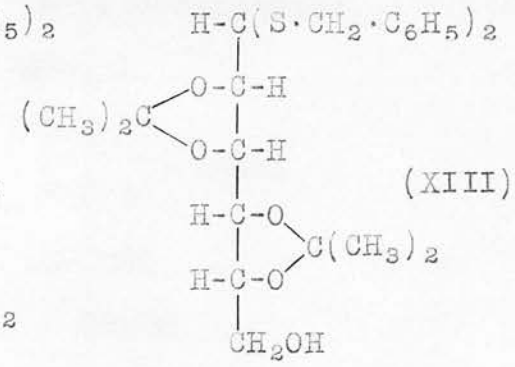
A larger proportion of diacetone compound (55%) proved to be present in the mixture of acetone compounds derived from mannose dibenzyl mercaptal than in the two previous cases, and with this increase in the proportion of diacetone compound present, there was noted a corresponding decrease in the yield of monomethyl mannose dibenzyl mercaptal. The methyl derivative isolated was recognised as 2-methyl mannose dibenzyl mercaptal, but it was always found in admixture with a larger quantity of mannose dibenzyl mercaptal itself.

The diacetone compounds of mannose dibenzyl mercaptal, which are possible, are (XII), (XIII), (XIV),

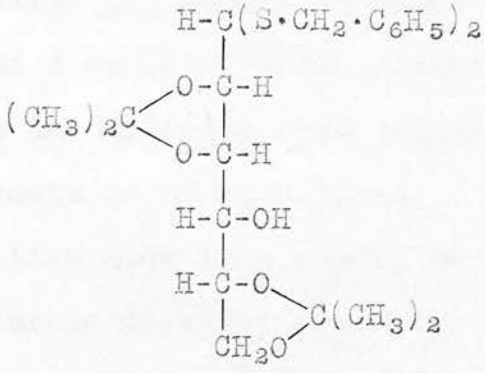
(XII)



(XIII)

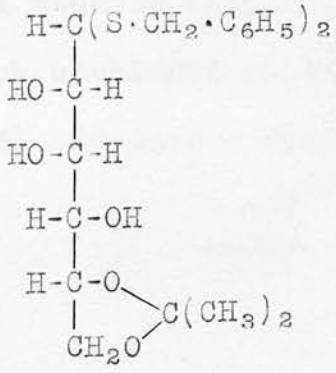


(XIV)

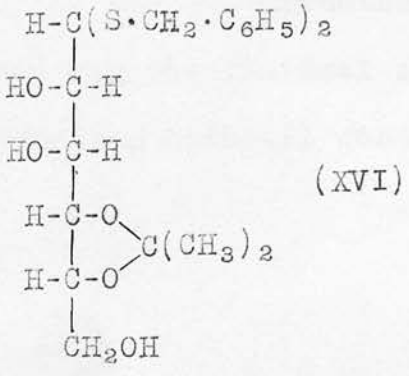


and of these (XIII) and (XIV) should form with greater ease than (XII). Yet, of these three diacetone compounds only (XII) could form a 2-methyl derivative on methylation. It might, therefore, be preferable to assume that the 2-methyl mannose dibenzyl mercaptal did not come from a diacetone derivative, but from a monoacetone, and of these latter compounds the three following forms are possible.

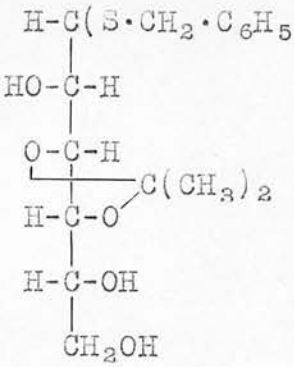
(XV)



(XVI)



(XVII)



The probability of acetone residues linking with hydroxyls 3 and 4 as in (XVII) can probably be eliminated, inasmuch as the molecule also contains a number of hydroxyl groups in cis-positions. By analogy with the supposition made with regard to the formation of 2-methyl glucose dibenzyl mercaptal from the monoacetone glucose dibenzyl mercaptal (II), it is necessary to assume that the reactive group in mannose dibenzyl mercaptal, as in glucose dibenzyl mercaptal, is the hydroxyl group in position 2, and that the methylation of a monoacetone compound such as (XV) or (XVI) would give the 2-methyl derivative. It is possible that the process of methylation employed was not sufficiently drastic to methylate such diacetone derivatives as (XIII) and (XIV), which would explain why the 2-methyl mannose dibenzyl mercaptal was contaminated with so much unchanged material, and why the residual syrup (p. 76) had such a comparatively low methoxyl content.

EXPERIMENTAL.

Preparation of the Acetone Compounds for Analysis.

The various acetone compounds were all prepared by condensing the crystalline dibenzyl mercaptal derivatives of the sugars with ten times their weight of dry acetone, containing concentrated sulphuric acid (3 c.c. per 100 c.c. of acetone). After 24 hours at room temperature the solutions turned brown, but on neutralising the acid with anhydrous sodium carbonate the brown colour disappeared. Filtration and removal of solvent at 50°/20 mm. yielded the acetone compounds in the form of very viscid, yellow syrups. It was possible to remove any unchanged original material by dissolution of the syrups in anhydrous ether.

The chief difficulty in preparing the acetone compounds for analysis was the complete removal of acetone condensation products. The acetone compounds were found to decompose completely at about 200°/0·03-0·05 mm. with the liberation of benzyl mercaptan, but partial decomposition, recognisable by the characteristically pungent odour of benzyl mercaptan, was noted at temperatures much lower than this. It was considered that 100°/0·05 mm. was necessary for removal of the last trace of acetone condensation products, but it is possible that even this treatment caused slight

decomposition.

A precipitation method, in which the acetone compound was dissolved in a little anhydrous ether and precipitated by the addition of light petroleum (b.p. 60°/80°), was attempted, but the high solubility of a number of the acetone compounds, notably that derived from glucose dibenzyl mercaptal, made this method impracticable.

The treatment which was shown to be the most satisfactory consisted in a preliminary heating at 100°/15 mm. for about 1 hour, followed by heating at 100°/0·05 mm. for 20 minutes. This method, while removing all acetone condensation products, was not sufficiently drastic to cause any appreciable decomposition.

Method of Acetone Estimation.

For the estimation of acetone a modification of Messinger's method (31), described in Cole's, Practical Physiological Chemistry (1933) page 355, was employed.

The acetone, liberated from the acetone compound by boiling with dilute sulphuric acid, was distilled into a freshly prepared solution of alkaline hypoiodite, when it is converted to iodoform. The alkaline solution was then acidified with hydrochloric acid and the excess of iodine estimated with standard sodium thio-

sulphate in the usual way.

(1) $I_2 + 2NaOH \longrightarrow NaOI + NaI + H_2O$.

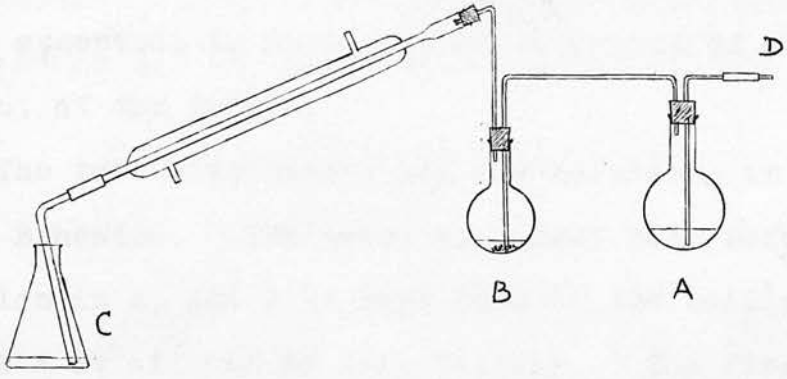
(2) $CH_3CO \cdot CH_3 + 3NaOI \longrightarrow CH_3CO \cdot CI_3 + 3NaOH$.

(3) $CH_3CO \cdot CI_3 + NaOH \longrightarrow CH_3COONa + CHI_3$.

From equation (2) it is seen that 1 gram molecule of acetone reacts with 3 gram molecules of NaOI, which, from (1), is obtained by the interaction of $3I_2$, or 6 litres of normal iodine, with NaOH.

Hence, 6000 c.c. of N. Iodine 58 g. acetone

or 1 c.c. of 0·1N. Iodine $\frac{5·8}{6}$ mg. acetone.



Method: The apparatus consists of two round bottom flasks A (300 c.c.) and B (100 c.c.) connected with a condenser as shown in the diagram. Flask A contains

50 c.c. of N-sulphuric acid, B contains 10 c.c. of water and a few glass beads and D is a glass plug closing the end of the outlet tube. To the condenser is attached an adapter which reaches to the bottom of a conical flask C (250 c.c.).

A quantity of the acetone compound, yielding approximately 12 mg. of acetone is accurately weighed out in a small glass tube, and the whole dropped into flask A. Then 10 c.c. of 40% sodium hydroxide are placed in flask C and 0·1N. iodine (25 c.c.) is run in from a pipette. It is important not to add the iodine to the sodium hydroxide until commencing the distillation, because sodium hypoiodite changes slowly to sodium iodate, which does not react with acetone, and it is essential to have present an excess of at least 10 c.c. of the iodine.

The tube D is closed and the solutions in flasks A and B heated. The water in B must boil before the solution in A, and B is kept just at the boiling point whilst A is allowed to boil briskly. The first appearance of turbidity in C is noted, and the distillation allowed to proceed for another 10 minutes, after which plug D is removed and the burners extinguished. The adapter is removed from the end of the condenser and washed with water into flask C.

The alkali in C is then neutralised by adding

carefully, with cooling, concentrated hydrochloric acid. An excess of acid is added and the liberated iodine estimated by titration with 0·1N. sodium thiosulphate.

A blank titration was carried out to ascertain whether the alkali had any action on the iodine, and careful testing of the apparatus showed that, when known samples, such as diacetone galactose or an acetone solution of known composition, were used for determinations, approximately theoretical results were obtained.

Preparation of the Acetone Compounds of Glucose Dibenzyl Mercaptal by the Copper Sulphate Method.

For this preparation the methods of Pacsu (3) were repeated. Glucose dibenzyl mercaptal (10 g.) was shaken for 48 hours with dry acetone (100 c.c.) and anhydrous copper sulphate (30 g.). The solution was filtered from copper sulphate and the solvent removed by evaporation at 15°/20 mm. The syrup was dissolved in a little chloroform and allowed to stand overnight at 0°, when any unchanged original material was deposited. This was separated, light petroleum (b.p. 60/80°) was added (2 volumes of petroleum to 1 volume of the

chloroform), and the solution allowed to stand for several days at 0°. A white crystalline precipitate (0·2 g.) appeared, and was separated and washed with petroleum. The crystals showed m.p. 92° (Cf. Pacsu (3).).

Analysis.

Found: $CH_3CO\cdot CH_3$, 12·1. Calc. for monoacetone glucose dibenzyl mercaptal, $C_{23}H_{30}O_5S_2$,

$CH_3CO\cdot CH_3$, 12·9%.

The filtrate, after removal of the crystalline precipitate, was concentrated to a syrup which was dried at 100°/0·05 mm.

Analysis.

Found: $CH_3CO\cdot CH_3$, 15·1%. Diacetone glucose dibenzyl mercaptal, $C_{26}H_{34}O_5S_2$ requires

$CH_3CO\cdot CH_3$, 23·7%.

Examination of the Syrup Remaining after the Removal of 4-Methyl Glucose Dibenzyl Mercaptal.

Methylation and hydrolysis of the acetone compound of glucose dibenzyl mercaptal gave two crystalline mono-

methyl glucose dibenzyl mercaptals and a syrup (p. 13 & 14). This syrup (9 g.), dissolved in 80% aqueous acetone (100 c.c.), was treated with a concentrated acetone solution of mercuric chloride (16 g.), as described on page 14, and the reducing sugar (3 g.) obtained as a syrup.

Analysis.

Found: OMe, 25·7. Calc. for dimethyl glucose, $C_8H_{16}O_6$,

OMe, 29·8%.

Methylation of Galactose and Mannose Dibenzyl Mercaptals with Methyl Sulphate and Sodium Hydroxide Solution.

Each of these mercaptal compounds (3 g.), dissolved in acetone (10 c.c.) and sodium hydroxide (4 c.c. of 30%), was methylated with methyl sulphate (8 c.c.) and sodium hydroxide solution (12 c.c. of 30%) during 1 hour at 60°. After heating for half an hour after the final addition of reagents, the mixture was poured into cold water, and the oil extracted with ether. The ethereal layer was washed with dilute sulphuric acid and water, dried over sodium sulphate, and evaporated to a syrup. Analyses of the syrups showed that

they both had methoxyl contents higher than required for monomethyl derivatives.

Analyses:

Found:

Galactose dibenzyl mercaptal derivative, OMe, 20·3.

Mannose " " " OMe, 10·0.

Calc. for monomethyl hexose dibenzyl mercaptal, $C_{21}H_{28}O_5S_2$, OMe, 7·3%.

Summary.

1. The analysis of the acetone compounds derived from the dibenzyl mercaptals of glucose, galactose and mannose is described.

2. From the results it is concluded that the monomethyl compounds are probably derived in each case from monoacetone hexose dibenzyl mercaptals, although the precise formulation of these compounds is in doubt.

3. It is shown that the dibenzyl mercaptals of 2-methyl glucose, 4-methyl glucose and 6-methyl galactose, on condensation with acetone, appear to yield only monoacetone derivatives.

In conclusion the author wishes to express his gratitude to Dr. E. G. V. Percival for his encouragement and valuable advice during the course of this work.

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This is to certify that Mr JOHN MUNRO, a candidate for the degree of Ph.D., successfully sustained an oral examination by a Committee of the Department on the subject of his thesis on 28 February, 1936.

Chairman of Committee.

8 May, 1936.